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HENRY FORD HOSPITAL MEDICAL BULLETIN

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MIGRAINE EQUIVALENTS*

LORNE D. PROCTOR, M.D.**

Migraine is an illness probably known to the Egyptians as early as 12 B.C., but first described adequately by the Greeks (Arateaus of Cappadocia)[1] in the first century A.D. Classically it applies to a vascular type of headache involving only one half of the cranium, preceded by a visual aura and accompanied by nausea and vomiting. The illness is recurrent and has a familial diathesis.

Wolff and his associates[2] have discussed vascular headaches extensively and have ample evidence supporting vasodilatation of blood vessels with an altered contractile state, probably following a phase of vasoconstriction, as the vascular dysfunction associated with migraine. The vessels involved are the internal and external carotid systems, the circle of Willis and the basilar systems. These phenomena may be seen at times in the conjunctival vessels and/or in the retinal vessels on the affected side.

There is little need to dwell on the so-called "migraine personality". May it be sufficient to point out that hypertonicity and rigidity of thinking and behavior are outstanding components in this personality pattern. It may be comforting to point out to migraine sufferers that this illness has a predilection for the more mentally agile individual.

Migraine and its equivalents follow, rather than accompany unusual stress.

In considering migraine equivalents, we will deal with those conditions in which apparently the above vascular dysfunctions produce clinical manifestations that do not conform to the classical migraine syndrome. The equivalent may be present as a cranialgia followed by some other neurological signs or symptoms or it may consist of a purely transient episode of a localized neurological dysfunction e.g., neuralgia, palsy, etc. Migraine equivalents, in my experience, are as common, if not more common than the classical migraine "attack".

In describing the various migraine equivalents it is easier to understand the variety observed if one considers the multitude of areas of the central nervous system that may be locally involved in this vascular dysfunction. Should only the occipital cortex be involved, quadrantic or hemiopic visual defects will occur without subsequent hemicranialgia or vomiting (ophthalmic migraine). If the parietal lobe is the site of this vascular dysfunction, paresthesiae, analgesia or various types of pain, usually limited to the face, neck and upper limbs on the contralateral side of the body, could be the predominant symptoms. Similarly if only the motor cortex is involved by the vascular dysfunction, facial weakness, monoplegia or hemiplegia could result. The sensory and/or motor dysfunctions may last for minutes or hours, and it is sometimes difficult to differentiate between such migraine equivalents lasting 12 to 48 hours and so-called "little strokes". Personally I am not quite certain what many authors mean by this latter term. I assume they have in mind at least a permanent occlusion of one or more small vessels producing this neurological clinical picture.

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If the migraine equivalent vascular dysfunction involves the temporal lobe (on the dominant side), particularly the posterior portion, episodes of expressive or receptive aphasia may occur. Extensive involvement of the temporal lobe or lobes could produce varying degrees of impaired consciousness. In this latter manifestation one must differentiate between a migraine equivalent and psychomoter epilepsy. This author[3] collected a series of 130 cases having as a common denominator a psychomotor variant EEG pattern, and among these more than 30% complained of episodic headache. Three of the 30% with headaches had the classical migraine type. Ninety percent of all cranialgias in this series improved on sodium dilantin medication. This is further evidence of the close relationship between migraine and epilepsy as first pointed out by Lennox[4].

When the uncinate lobes are involved in this vascular process, gustatory and/or olefactory episodes may occur. A patient seen by the author had suffered classical migraine attacks for many years and these were replaced in her 40's by periods of smelling "strong gasoline". The most effective therapy for these attacks continued to be Cafergot, the same medication she had used for the previous migraine episodes. The usual gustatory experience is an episode of metallic or sour taste.

Continuing consideration of various cortical areas involved by the "migraine" type of vascular dysfunction, one of the more rare equivalents, manifesting an episode of spatial disorientation — the patient feels suspended above the floor, not necessarily in the vertical position and is confused as to right and left, etc. — is thought to be due to localization of the vascular dysfunction in the region of the angular gyrus[5].

Much has been written about abdominal migraine, a migraine equivalent. Headache may precede the abdominal cramps, nausea and possibly vomiting, or abdominal migraine may not include the "aura" headache. The insula area may be involved in these episodes. Some authors[5] have described episodes of thoracic pain, pelvic pain or precordial pain, the latter particularly associated with tachycardia and dyspnea (precordial migraine). Nausea and vomiting may precede or accompany such episodes. I find it difficult to suggest the probable localization of the central nervous system dysfunction in these equivalents.

Another rare equivalent is episodes of cerebellar ataxia, no doubt the result of "migraine" vascular dysfunction involving the cerebellar arteries. Similarly, basilar artery involvement could produce episodes of vertigo, impairment of conjugate deviation of the eyes, ataxia and finally even impairment of consciousness, e.g., confusion, etc., through involvement of midbrain reticular formation.

Severe autonomic or hypothalamic migraine equivalents are also rare, but the milder forms are more frequently seen, in which periodic episodes of hypersalivation, rhinorrhea, dilation of pupils, hyperhidrosis and flushing preceding or attendant with headache occur.

The author has not observed ophthalmoplegic migraine, another equivalent, but Merritt and Friedman[1] describe cases with episodes of recurrent unilateral headache accompanied by extraocular palsies. They suggest pressure upon the 3rd cranial nerve passing between oedematous superior cerebellar and posterior cerebral arteries,

and similarly pressure on the 6th cranial nerve passing between oedematous auditory and inferior cerebellar arteries as the basis for these extraocular palsies. The 3rd cranial is the most commonly affected (10 times), and the pupil invariably is dilated. The palsies may last for days or weeks after the headache.

Recurrent episodes of a variety of neuralgias, with or without headache, could be included in this ill defined equivalent category. The most common involves the trigeminal area, probably through vascular embrassment of the Gasserian ganglion. The remaining facial neuralgias in this group could be attributed to a "migraine" dysfunction of the external carotid artery, and would include Gardner's[6] petrosal neuralgia, vidian neuralgia and appears to closely resemble Horton's[7] histaminic cephalalgia and cluster headaches.

Finally, neuralgia over the distribution of the temporal artery is mentioned only to set out that transient changes in and about this vessel are associated with this complaint. Wolff[2] has used the changes in tissues surrounding the temporal artery in such a case to illustrate that oedema and change in pain sensitivity does occur in subjects with vascular headaches of the migraine type. Such a neuralgia should be differentiated from temporal arteritis. This latter "neuralgia" is progressively of longer duration, and the artery is found to be hard and nodular and exquisitely tender.

Bell's palsy has been noted as an accompaniment of headache, and may last for hours or days after the headache.

The differential diagnosis of migraine equivalents must include tension headaches, the most common of all headaches (30%), but this presents little difficulty as the occipital muscle mass is usually tense and tender to pressure, the headaches are not cyclic, but continuous (days or weeks) and nausea or vomiting seldom occurs; there is no aura.

It is most important to keep in mind that migraine equivalents may be symptomatic of organic rather than functional disease of the central nervous system, and should there be any question as to the cyclic nature of the dysfunction or if there occurs a progressive increase in severity of consecutive episodes, a most thorough neurological investigation, including angiography, pneumoencephalography and electroencephalography is indicated. The lack of response, of what is thought to be migraine equivalent, to early treatment by vasoconstrictor drugs should arouse a strong suspicion regarding this diagnosis.

In the case of precordial pain, the usual thorough cardiovascular investigation is indicated before the diagnosis of migraine equivalent is made as would be a thorough gastroenterological investigation in the case of abdominal magraine. Electroencephalography will assist in screening out those borderline cases of so-called epileptiform syndromes where the migraine syndrome is in reality an epileptic equivalent, and requires anti-convulsant medication, i.e., sodium dilantin and phenobarbital, rather than vasoconstrictor drugs.

The treatment of migarine equivalents is the treatment of migraine. Prophylaxis is important. We are usually dealing with hypertonic individuals that are experiencing

difficulty adapting to their stress. The need for psychotherapy is obvious, and many can be adequately treated by merely supportive psychotherapy with adjustment of habit pattern and a mild relaxant e.g., sodium amytal, grs 1, b.i.d. or t.i.d. Others may require interpretive psychotherapy or a lengthy formalized psychiatric program. Alcohol ingestion is one of the best ways to trigger a migraine equivalent.

During the attack ergotamine tartrate, 1 mgm, with caffeine, 100 mgms (Cafergot) has been found to be of the greatest value. It should be administered at the earliest possible stage of the attack, and if the patient cannot tolerate this medication orally (vomiting), suppositories, 2 mgms ergotamine tartrate with 100 mgms caffeine, may be used. The maximum dose of ergotamine should be 6 to 8 mgms per day, and this program not repeated in less than 10 days. If parenteral ergotamine is necessary, dihydroergotamine methanesulfonate (DHE 45) is indicated. It is less likely to produce side effects e.g., vomiting, cramps, tingling of hands, etc., but as it has less vasoconstriction action than ergotamine tartrate a 1 mgm dose intramuscularly could be repeated in 1 hour. Ergotamine compounds are contra-indicated in the presence of pregnancy, peripheral vascular disease, angina pectoris, impaired renal or hepatic function, etc.

Analgesics e.g., acetylsalicylic acid and its combinations including, if necessary, a minimum dose of codeine is the usual means of controlling a lengthy cranialgia.

There are many other pharmacological preparations for the treatment of migraine and the equivalents, e.g., wigraine, migral, histamine diphosphate, diamox, octin hydrochloride, etc. A very thorough consideration is given to this subject in Merritt & Friedman's recent text.[1]

The medical problem of migraine equivalents lies in the confusion these syndromes create if the internist does not consider the vascular nature of their aetiology. They may mimic, in part, intracranial disease of many types, i.e., neoplastic, vascular, the neuritides, meningitides, Meniere's disease, arteritis and epilepsy, etc. The cyclic nature of their occurrence, the usually episodic nature of this syndrome and the marked benefit from vasoconstrictor drugs will be of the greatest diagnostic assistance. If we are to diagnose migraine equivalents, we, of course, must think of them, remembering they may be symptomatic of organic disease of the central nervous system.

REFERENCES

1. Friedman, A. P., and Merritt, H. H., eds.: Headache, Philadelphia, F. A. Davis, 1959.

2. Wolff, H. G., Tunis, M. M., and Goodell, H.: Studies on headache; evidence of tissue damage and changes in pain sensitivity in subjects with vascular headaches of the migraine type, A.M.A. Arch. Int. Med. 92:478, 1953.

3. Proctor, L. D.: Effect of various medications on patients manifesting an epileptiform syndrome, Am.. J. Psychiat. 104:380, 1947.

4. Lennox, W. G.: Treatment of epilepsy and of migraine, New England J. Med. 222:657, 1940.

5. Vance, F. V., and Klingman, W. O.: Subtypes of migraine, Virginia M. Monthly. 79:363, 1952.

6. Gardner, W. J., Stowell, A., and Dutlinger, R.: Resection of the greater superficial petrosal nerve in the treatment of unilateral headache, J. Neurosurg. 4:105, 1947.

7. Horton, B. T., MacLean, A. R., and Craig, W. McK.: A new syndrome of vascular headache; results of treatment with histamine; preliminary report, Proc. Staff Meet., Mayo Clin. 14:257, 1939.

PHEOCHROMOCYTOMA — A SURVEY OF CURRENT CONCEPTS

HENRY L. GREEN, M.D.*

A considerable amount of information is now available about the physiology, clinical behavior, and management of pheochromocytoma. Unfortunately, much of this data is so widely scattered through the literature that it is accessible only by time-consuming search. The author has attempted to bring it together. A few of the less common features are included for their interest. No pretense of exhaustiveness is made, although many of the articles referred to are themselves reviews.

HISTORY

The first pathological description of what was probably a pheochromocytoma was published by Fränkel in 1886.[13] Labbé[22] is credited with recognition of the syndrome of paroxysmal hypertension associated with these tumors. Ante-mortem diagnosis was first accomplished by Vaquez in 1926.[44] Charles Mayo first excised such a neoplasm, apparently without realizing what it was.[32] In 1929, Pincoffs was able to predict the presence of a pheochromocytoma, the diagnosis being later confirmed at surgery.[36]

TERMINOLOGY

The terms *pheochromocytoma* and *chromaffinoma* refer to the functioning tumor of the adrenal medulla. They may also be used to designate ectopic chromaffin tumors. *Paraganglioma* is used synonymously by some authors, while others apply it only to the extra-adrenal chromaffinomas. Formerly it was thought that the carotid body tumor and related neoplasms were also paragangliomas. These have since been shown to be neither chromaffin positive nor of ectodermal origin. Carcinoid tumors have also been considered paragangliomas in the past.[39]

INCIDENCE

Approximately 400 cases of pheochromocytoma have been reported. This figure belies their true incidence. For example, Graham[15] found 8 pheochromocytomas in the course of 1700 sympathectomies performed on hypertensive patients (0.47%).

The sex incidence is about equal, except in childhood when boys predominate.[3,24] It is found at any time of life, from infancy to old age, but most often between 20 and 40 years.[3,20,39] A familial occurrence has sometimes been noted, especially for tumors involving the organs of Zuckerkandl, and for those associated with neurofibromatosis.[5,17] About one tenth of the reported cases have been in pregnant women.[37]

PATHOLOGY

Pheochromocytomas may develop wherever chromaffin tissue occurs, either embryrologically or in the adult.[15] The commonest location is in or about the adrenal (82%), particularly the right one.[21,39] They also found along the cerival, thoracic, or abdominal sympathetic chain.[20] In the chest they may be seen roentgenographically as paravertebral masses even before the onset of symptoms.[39] They are also located along the major vessels of the abdomen (presumably arising from the organs of Zuckerkandl and other bodies) and the mesentery of the small intestine.[21,39] Very rarely they occur in the urinary bladder,[23,28] or intracranially.[48]

*Division of Cardiovascular Disease

About 10% are bilateral.[17] In children both multiple and extra-adrenal tumors are considerably more common.[34]

Their size varies from the microscopic (detectable only by the effect of surgical manipulation of the affected adrenal)[8] to huge masses weighing up to 8 pounds.[4,39,48] The majority weigh less than 100 grams.[48] At operation a peculiar brown pigmentation of the perirenal fat may be seen.[15] Benign chromaffinomas are well encapsulated, compressing but not invading the neighboring organs. Cystic and necrotic areas in the tumor reflect its great metabolic demand. Calcification is sometimes seen, even on x-ray.[2,5,39]

Microscopically the architecture is variable even within the same tumor. Nests, trabeculae, cords or other arrangements of cells lie in the highly vascular stroma. The cells are large but tend to resemble those of the adrenal medulla. Lipochrome and brown pigment are seen in the cytoplasm in chromate fixed specimens. Multiple infarcts are common.[5,39]

The affinity for chrome salts has been directly attributed to the catecholamine content of the tissue. Chemical analysis of chromaffin tumors has repeatedly confirmed this correlation. However, a patient was recently cured of hypertension by surgical removal of a *non-chromaffin* paraganglioma from the aortic bifurcation. Analysis of both the patient's urine and the tumor revealed large amounts of catecholamines.[33]

About 10% of pheochromocytomas are malignant.[5,17,20] The incidence is higher for bilateral tumors and for those associated with persistent hypertension.[21] Metastases occur to liver, lungs, bones, regional lymph nodes, and other organs.[5] As with other endocrine tumors, malignancy is very difficult to assess histologically, and those originally considered benign may later be found to have metastasized.[39]

PATHOLOGIC PHYSIOLOGY

The clinical features associated with these tumors are due to their secretion of biologically active chemicals and to their mechanical effects. In most cases the hormones produced are mainly epinephrine and norepinephrine. The latter usually accounts for the greater fraction.[9,50] However, neoplasms containing large amounts of 3-hydroxytryptamine and DOPA have been described.[46]

There is only a crude relationship between the chemical nature of the secretions and the clinical picture produced. Pharmacologically, epinephrine is known to raise the systolic blood pressure and pulse rate. Elevation of the blood sugar, hypermetabolism, apprehension, mydriasis, and other phenomena are also produced. Norepinephrine increases both systolic and diastolic blood pressure, slows the pulse, and has far less effect on the other body processes. Thus a small chromaffinoma producing mainly norepinephrine gives rise to the clinical picture of essential hypertension, while epinephrine secreting tumors are apt to be associated with nervousness, palpitation, diabetes, etc. However, large doses of epinephrine will elevate the diastolic pressure, and large doses of norepinephrine produce metabolic symptoms. The ratio of the concentrations of the two hormones also seems to play a part.[5,9,45] Following

the removal of a malignant pheochromocytoma its metastases may continue to elaborate pressor substances.[45]

In the metabolism of epinephrine and norepinephrine, they are apparently converted into their 3-methoxy derivatives. Unlike their parent compounds, these substances are effective substrates for monoamine oxidase. This enzyme converts them into 3-methoxy-4-hydroxy mandelic acid (vanillylmandelic acid, V.M.A.). This is excreted in the urine along with the glucuronides of 3-methoxyepinephrine and 3-methoxynorepinephrine. Only a small percentage of the epinephrine and norepinephrine is excreted unchanged.[50]

CLINICAL FEATURES

An impressive variety of syndromes may be produced in different patients. In 70% of cases there is sustained hypertension. Upon this there may or may not be superimposed paroxysms of increased blood pressure and metabolic activity. Less often the "classical picture" is seen, with normal blood pressure between crises.[35,39,45,48,49] Almost any of the symptoms may so dominate the illness as to make the diagnosis obscure. Recurrent vomiting,[14] abdominal symptoms,[14] or night sweats[47] may be the presenting difficulty. The patient may develop a myocardial infarction, a stroke, or pulmonary edema without ever having had previous evidence of the tumor.[18,21] On the other hand, asymptomatic pheochromocytomas have been discovered during surgery for some unrelated condition.[48] Usually the patient has had difficulty for a period of 6 weeks to 10 years.[21] Moore[34] has emphasized the great difficulty of eliciting the symptoms even when they are present.

In childhood the picture is about the same as in the adult, except that hypertension is almost invariably sustained.[34]

Table 1 presents a list of the commoner symptoms in approximate order of frequency.[15] Obviously the variations which may occur from patient to patient are innumerable.

Table 1

Common symptoms of pheochromocytoma

Headache (usually pulsating, severe)	Pallor or flushing of face
Palpitation	Dizziness
Vomiting	Substernal pain
Sweating	Abdominal pain
Dyspnea	Nervouseness or anxiety
Weakness	Nausea

Other symptoms include pain and numbness of the legs,[21] tingling and coldness of the hands and feet,[21] and blurred vision or even blindness.[35] Subjective and objective coldness associated with vasoconstriction is a typical feature.[11] Raynaud's phenomenon has been described.[27]

Crises may be precipitated by a wide variety of circumstances, commonly involving some change of posture or other mechanical stimulus to the tumor, (Table 2). Wingo[48] has called attention to the fact that many of these stimuli involve a small initial reduction in blood pressure. He postulates that the attack may represent an inappropriately dramatic response of the tumor to this hypotension.

Table 2

Factors Precipitating Paroxysms

Change of posture (bending, stooping, lateral flexion)	Alcohol	Sleep
exertion	histamine (as in gastric analysis)	laughing
trauma to side or abdomen	amobarbital sodium sedation	sexual intercourse
local heat	TEA chloride	shaving
perirenal air insufflation	pain	gargling
general anesthesia	hyperventilation	straining at stool
parturition	emotional stress	sneezing
meals	postural hypotension	having BP taken
	carotid sinus pressure	urination (pheochromocytoma of bladder)
	change of temperature	

The frequency of attacks ranges from several dozen a day to one every few months. They last from seconds to hours, commonly 10 or 15 minutes. They tend to increase in frequency but not severity. While the patient is often exhausted following paroxysms, he may be well between them. Some experience isolated symptoms, varying in severity, beginning and ending gradually, and lasting hours or days. There may be continuous hypermetabolism and/or hyperglycemia with paroxysmal hypertension. Severe attacks may end fatally.[9,20,21,24]

As stated, the blood pressure is usually elevated continuously, but paroxysms may be superimposed. Following an attack, the pressure may return to previous levels or hypotension of various degrees may occur. Dybkœr[9] feels that adaptation of the body to the high concentration of pressor substances accounts for the various sequelae of an attack. When secretion diminishes slowly, the blood pressure gradually falls to its former level. With more abrupt fall in concentration, it drops below that level, but returns spontaneously. A sudden cessation of secretion may result in shock or even death.

Either systolic or diatolic blood pressure or both may be raised. The pulse rate may be fast or slow. Postural tachycardia and less reliably postural hypotension have been considered of diagnostic importance.[15,41]

Some degree of fever is common, probably because of hypermetabolism coupled with impaired heat dissipation due to vasoconstriction. Even hyperpyrexia may occur, especially in the terminal stages.[11,25,41]

Characteristically the patient is thin.[21] Shivering is not uncommon.[9] When combined with sweating, a striking picture is produced. There may be pallor, flushing, or mottling of the face.[9,15,18,21,35] The peripheral arteries may be constricted, even to the point of disappearance of the pulses.[35] The pupils may be dilated.[9] The fundi often reflect the degree of hypertension.[15] In addition the retinal vessels may show constriction.[35]

An abdominal mass, either the tumor itself or a displaced liver or kidney, is palpable in 15% of cases. Even when it is not, palpation or massage of the flank may trigger an attack, if the tumor is sufficiently large. This sign should be deliberately sought. A syringe of phentolamine is kept ready for emergency use. If a

paroxysm is produced, termination of it by injection of the drug will also be of confirmatory value.[5,20,35]

Certain diseases seem to show a peculiar association with pheochromocytoma. Five percent of the patients have neurofibromatosis.[5] Von Hippel-Lindau's disease (angiomatosis retinae and cerebelli) may occur along with the neoplasm.[49] There appears to be an increased incidence of gallstones.[21] Rarely, Cushing's syndrome is a concomitant.[15,18]

DIAGNOSIS

There are many circumstances that should call to mind the possibility of pheochromocytoma. Each author emphasizes a somewhat different set of indications for applying the screening tests. They may be summarized as follows:[2,3,18,20 21,24,25,49]

1. In the hypertensive
   a. paroxysmal hypertension or wide fluctuation within the hypertensive range
   b. hypertension in young individuals, pregnant or puerperal women, or in the absence of positive family history
   c. hyptersion of recent onset
   d. severe hypertension
   e. hypertension with a normal pressor response
   f. hypertension associated with a postural fall in blood pressure and/or rise in pulse rate
   g. paradoxical response to ganglionic blocking agents
   h. hypertension associated with diabetes, hypermetabolism, fever, sweating or neurofibromatosis
2. A rise in blood pressure associated with general anesthesia, when no other cause is evident. If this occurs, the procedure should be discontinued until pheochromocytoma has been ruled out, as surgery for unrelated conditions carries a 50% mortality in the presence of such a neoplasm
3. "Seizures" characterized by any combination of the symptoms previously mentioned, especially if precipitated by factors such as those listed in Table 2
4. Nervous or hypermetabolic symptoms
5. Headache
6. Shock following the administration of a phenothiazine drug

Kvale states that it is probably rarely necessary to consider the diagnosis in an obese patient.

DIFFERENTIAL DIAGNOSIS

1. *Hypertension due to other causes.* While the hypermetabolic, paroxysmal, and other features of pheochromocytoma are of diagnostic value, their absence does not exclude the diagnosis.[15,17,49] In sustained hypertension due to chromaffinoma, the duration of illness is usually much shorter than in essential hypertension. This is not invariable, however, and the other features of the two may be identical. Probably almost all hypertensives should be

screened for pheochromocytoma. As seen, the syndrome must also be distinguished from the toxemias of pregnancy.[1,37]

2. *Hyperthyroidism.* Paradoxical weight loss, apprehensiveness, weakness, fine hair, restlessness, and fine tremor may all be caused by pheochromocytoma. The BMR is often significantly elevated. At times thyromegaly and exophthalmos compound the difficulty. The blood cholesterol, protein bound iodine, and radioactive iodine uptake usually help distinguish this syndrome from thyrotoxicosis, but reportedly the PBI ca nbe elevated in pheochromocytoma. Antithyroid drugs are usually fairly ineffective, but they may cause a temporary reduction in BMR.[7,17,49]

3. *Diabetes.* During paroxysms elevated blood sugar with diminished glucose tolerance are common. Insulin resistant diabetes and even acidosis have been noted, with complete clinical disappearance of the diabetes following removal of the pheochromocytoma. Any diabetic with severe hypertension or with retinopathy characteristic of hypertension should be investigated[10,20,-24,25,40,50] Kimmelstiehl-Wilson disease may be mimicked.

4. *Headache* may be the dominant feature. This may resemble a tension headache or one of the vascular types, such as migraine or histamine cephalalgia..[10,20,40]

5. *Other neurological disorders,* including stroke, brain tumor, epilepsy, tabes dorsalis, and "acute nervous system infection or allergy" must be differentiated.[15,18] In children the first impression is frequently that of an intracranial lesion.[34]

6. *Psychosis or neurosis* may be the presenting difficulty. This may take the form of anxiety or hysteria, often with hyperventilation. It may also resemble menopausal tension, agitated depression, or cyclothymic psychosis. Hypertension may not be detected.[18] Electroshock therapy or other procedures in such patients may, of course, be disasterous.

7. *Coronary artery disease,* ranging from coronary insufficiency to myocardial infarction may be imitated or may actually co-exist.[10,19,20]

8. *Gastrointestinal symptoms* may not be very characteristic or may be such as to lead to the diagnosis of an acute abdominal emergency. Shock and ileus may accompany severe abdominal pain. Infarction of the tumor is thought to account for acute pain, whereas chronic symptoms may be caused by pressure on the surrounding structures. Again, there may be no evidence of previous or concomitant hypertension.[5,14,19]

9. *Miscellaneous conditions* to be differentiated include the following:[15,18,49]

lead poisoning	gastrointestinal hemorrhage
hyperinsulinism	urticaria
various renal lesions, including glomerulonephritis	angioneurotic edema

LABORATORY FINDINGS

The routine laboratory tests are not very helpful.[15,18,20,49] Albuminuria occurring after attacks or continuously is found in about two-thirds of cases. The blood sugar is often elevated; in fact the patient may be frankly diabetic. Serum potassium is elevated, especially with a paroxysm, owing to the action of epinephrine. The electrocardiogram may show the changes expected with hypertension due to any cause. A basal metabolic rate as high as 90 or 100 may be found. While classically normal,[41] the cold pressor test has been known to be positive, or even to trigger a crisis.[11,49] Kvale[22] states that he has not observed this however. The test is almost always abnormal in essential hypertension. Intravenous pyelography may demonstrate a mass.

PHARMACOLOGIC TESTS

If carefully selected and applied, these are valuable screening procedures. Their value is limited somewhat by the lack of uniform criteria for the normal limits of many of the tests.

There are two general types. Blocking tests are used if the patient's blood pressure is found to be elevated (e.g. over 170/110[22]). A substance is administered which blocks the effect of circulating pressor amines. Provocative tests are intended to cause a paroxysm in a patient whose blood pressure is normal or only slightly elevated[45] (Kvale says under 170/110). If the pressure fluctuates widely, both types of tests should perhaps be done at appropriate blood pressure levels.

Certain precautions will enhance the reliability of these procedures. Obviously one could easily be misled by a difference between the blood pressures in the two arms. Errors due to spontaneous fluctuations in the pressure should be minimized by following the basal blood pressure for at least 30 minutes before the test, or at least by having the patient rest for a similar period. Similarly the blood pressure should be followed for at least 15 minutes after it has returned to the control level. A cold pressor test should be done before giving any provocative agent to avoid misinterpreting the results obtained.[20,35]

Many medications are capable of causing false positive or false negative results (see below).

Phentolamine (Regitine) methanesulfonate test: Following rapid intravenous injection of 5 mgs. of the drug, the blood pressure is taken every 30 seconds for 5 minutes, and then every minute for 15 or 30 minutes, or until it stabilizes at control levels. A positive result consists of a fall of 35 mms. of systolic and 25 mms. in diastolic pressure. This usually begins immediately, reaches a nadir in 2 to 5 minutes, and lasts 10 minutes to an hour or more. Occasionally, a "fall-rebound-fall" response occurs, which also represents a positive test. A fall the first minute, with return to basal levels in the next minute is negative.[20,35]

False positive phentolamine tests occur in subjects receiving narcotics, sedatives, rauwolfia compounds, or thiocyanates, and in uremic patients. Sedatives and narcotics should be stopped for 48 hours, thiocyanates 4 to 6 days, and rauwolfia several weeks prior to the test. With these precautions, false positive tests are allegedly rare,[18,20,21,37,49] although some[45] feel they are not unusual.

False negative tests may be seen in patients being treated with hydralazine and other antihypertensive drugs. Hydralazine should be discontinued for several weeks,[20] and other drugs for several days.

An intramuscular phentolamine test has also been used, but is probably less reliable.[35]

Most workers consider Regitine the blocking drug of choice, as it is quite safe. The side effects are minimal in the recommended dose, consisting of tachycardia, weakness, dizziness and flushing. The test can even be performed on an emergency basis in the presence of hypertensive encephalopathy.[20] To this author's knowledge, two fatal reactions have been reported.[10,37] Lance[24] feels Regitine may be dangerous in patients with normal basal blood pressures.

Piperoxan (Benodaine) hydrochloride test. This blocking test is performed by giving 0.25 mgs. per kg. (the maximum 20 mgs.) of the drug intravenously over a 2 minute period. The blood pressure is followed exactly as in the phentolamine test. Criteria for a positive test are less well established, but a fall of 35 mms. in systolic or diastolic pressure has been suggested. Again, a "fall-rebound-fall" response is positive.

False positive and negative results probably occur under the same circumstances as in the Regitine test. The drug is not as safe as phentolamine, and side effects may be serious. In essential hypertension, tachycardia, headache, flushing, anxiety, chest or abdominal pain, nausea, vomiting, and dizziness may be seen. In these patients the blood pressure may fall initially, but often rises, at times to dangerous levels. Encephalopathy, convulsions, and pulmonary edema can occur. For this reason Orgain[35] does not recommend the test for routine screening.

An intriguing new test, the *piperoxan antidiuresis test,*[6,26,31] makes use of the fact that the drug abolishes the diuretic effect of pressor amines. This action is even more marked than that on the blood pressure. After an overnight fast the patient drinks a liter of 0.25% sodium chloride solution in 10 minutes. He remains recumbent, and smoking is prohibited. After voiding, urine is collected at half hour intervals for 3 or 4 periods. Immediately following the second voiding, 20 mgs. of piperoxan are injected intravenously over a two minute period. Subsequent urine collections show a prompt reduction in volume, generally one-third or less the previous levels, in patients with pheochromocytoma. Control patients actually showed a slight increase in urinary output. The test is said to be positive even during the normotensive phase of the disease.

The *dibenamine hydrochloride test* is relatively more dangerous and less reliable than the other blocking procedures and is little used.[5,35,45,48,49]

Histamine test.[20,21,25,48] After stabilization following a cold pressor test, 0.025 to 0.10 mgs. (usually 0.05 mgs.) of histamine base are administered intravenously in about 0.5 c.c. saline with a tuberculin syringe. Regitine is kept ready for emergency use. Blood pressure is determined every 30 seconds for 5 minutes, and then every minute for 15 minutes or until it returns to the control level. If the drug was

injected into a vein, the blood pressure falls after 30 seconds. If the test is positive, it then rises rapidly, 60/35 mms. or more higher than the cold pressor response. This rise occurs one to four minutes after injection and lasts 5 to 15 minutes. Symptoms of a severe paroxysm appear.

The mechanism of action is not known. It may be direct stimulation of the tumor, or the paroxysm may be a response to the initial hypotension produced.

This is generally regarded as the most reliable provocative test. False positives are seldom seen,[18] but may occur with larger doses. It may be falsely negative in patients receiving sedation or anesthesia, or if the control pressure is too high, or if the drug is injected slowly.

There is some danger in the procedure. The blood pressure may fall perilously in the absence of a chromaffinoma, or climb to alarming levels in the presence of one.[45] A number of deaths have been reported.[47] Nevertheless, the test is probably indicated in all pheochromocytoma suspects who have a normal or fluctuating blood pressure.

The *methacholine (Mecholyl) chloride, tetraethylammonium chloride,* and *DMPP* provocative tests have little to recommend them.[18,20,35,45,48]

Epinephrine tolerance test. This is rarely used. It is based on the decreased response to epinephrine shown by patients with pheochromocytoma, probably due to an adaptation phenomenon.

CATECHOLAMINE ASSAY

Improved and simpler techniques of catecholamine assay are continually being perfected. The directness of this approach is a clear advantage. Some even believe that where this method is available the pharmacologic tests are seldom indicated.[45]

Urine assay is most commonly done. A normal resting individual excretes 15-30 mcg. per 24 hours, 80% of which is norepinephrine. It never exceeds 185 mcg.* In the presence of pheochromocytoma, the range is from 180 to 9000 mcg. The increase is usually in the norepinephrine fraction, but can also be epinephrine.[16] Bananas contain large amounts of norepinephrine and should be withheld for the test.[49] Rauwolfia drugs should be stopped for a month prior to urine collection. Many other substances may interfere with the determination depending on the technque used.[51] The output is generally high even during normotensive intervals,[9,35] but may be normal at these times.[20,28] If normal, it may reach diagnostic levels under the influence of histamine or general anesthesia.[5]

Although the test is believed to be highly specific, catecholamine output is also somewhat increased in the following conditions:[30]

asphyxia	emotional stress
cold exposure	electroshock therapy
myocardial infarction	increased intracranial pressure

Numerous quantitative and semi-quantitative techniques are used, including bioassay, paper chromatography, photofluorimetry, and chemical analysis.[16,21,35]

*At the Henry Ford Hospital the normal range is up to 300 mcg. total catecholamines per 24 hours.[51]

A new method determines the urinary output of VMA (see Pathologic Physiology, above). It may be a better test, for this metabolite is excreted far more abundantly than are the pressor amines themselves.[40,50]

Blood catecholamine assay is also useful. It is apparently unnecessary to withhold drugs for the test. The level is considered significant when over 3.5 mcg. of epinephrine-like substance per liter of plasma is found. It can be normal in the presence of a pheochrome tumor,[26] but will be found elevated if the blood is obtained during a histamine-induced paroxysm.[20,21] In essential hypertension, the level is usually less than 3.5 mcg. per liter and does not rise significantly after histamine. The plasma catecholamine content also rises in some patients with renal insufficiency, increased intracranial pressure and lymphoma. It is uncertain whether the substances measured in these conditions are the same as those produced by chromaffinomas. With the fluorescent column technique, hemolysis or icterus will produce falsely high results. Users of epinephrine nebulizers may also have elevated blood catecholamine levels.[20,21]

LOCALIZATION OF THE TUMOR

Because pheochromocytomas occur in so many locations and may be multiple, many authors do not consider exact preoperative localization essential. Rather than subjecting the patient to potentially dangerous procedures, the surgical approach is such as to permit rather wide exploration. If the plain abdominal x-ray and intravenous pyelogram are non-contributory, operation is not delayed.[20,24,35]

Others feel that exact localization is highly desirable. Aortography, presacral or perirenal oxygen insufflation, retrograde pyelography, selective aortography, and tomography have been employed. The gas insufflation techniques, at least, are not without hazard. Another very ingenious method has been devised, whereby blood is sampled from various parts of the venous system using a catheter under fluoroscopic control. The relative catecholamine content of the specimens helps localize the tumor.[45]

Interestingly, tumors producing both norepinephrine and epinephrine tend to lie in connection with one of the adrenal glands. Those elaborating norepinephrine alone are usually ectopic.[45]

TREATMENT

The only successful form of treatment is surgical removal of the tumor. X-ray therapy has been disappointing.[15]

Preoperatively it may be necessary to control dangerous swings in blood pressure.[43] Phentolamine methanesulfonate is apparently of great value here. In one case[42] a continuous infusion of the drug not only prevented the hypertensive crises, but eliminated the intervening periods of shock. Surgery was then carefully planned and carried out two and a half days later. These workers used a concentration of 30 mgs. per liter, given intravenously at 20-80 drops per minute, depending on the intramuscular frequent blood readings. Intermittent Regitine had been unsuccessful in their patient. On the other hand, Emanuel et al[10] warn that the drug does not seem to protect humans from arrhythmias induced by pressor amines.

The operating team should be well prepared for the emergencies that are apt to arise. Continuous monitoring of the blood pressure by an intraarterial cannula is probably worth while. The anesthetic agent and body position must be chosen carefully.[24,45] When anesthesia is induced and the incision is made, blood pressure rises to various degrees. Five mgs. of Regitine IV may be advisable at this time. Various surgical approaches are used. A high transverse abdominal incision permits wide exploration.[20]

The tumor should be treated gently and its vessels ligated early. As it is palpated, 5 mgs. or more of Regitine may be used to control paroxysms.* With tying of the vessels, shock often occurs. This is controlled by the administration of pressor agents sufficient to maintain the blood pressure at about 100/70 to 110/80.[20] It is probably even advisable to make the fall a gradual one by giving these drugs relatively early.[9] If no drop occurs, the surgeon should search for additional tumor tissue.[20] Preservation of some adrenal cortex is important. If this cannot be done, intravenous hydrocortisone should be at hand.[20]

After surgery Thorne[43] recommends leaving the patient undisturbed on the operating table for several hours. Extremely close observation is still necessary, for the blood pressure may drop unexpectedly.[20] Urinary catecholamine determination will help assess the completeness of the operation.[45]

PROGNOSIS

Untreated pheochromocytomas are generally fatal in a few years, their course resembling that of malignant hypertension.[45] Death is usually caused by pulmonary edema, coma, cerebral hemorrhage, or shock during surgery or other circumstances.[12,20] The author is aware of one report of fatal hemorrhage into a previously asymptomatic tumor.[25]

When found in relation to pregnancy, the disease may be fatal before or after parturition, often within a day of delivery. The overall maternal mortality has been about 50%, while that for the infant is approximately 40%.[1,3,37]

The fatality rate in childhood is about 54%.[34]

Surgical removal of the tumor often results in cure. Partial or complete reversal of the changes in the circulatory system may result. If hypertension persists, the possible causes include the presence of additional tumors or metastases, irreversible vascular damage due to previous hypertension, and the presence of essential hypertension. Operative mortality has been 20 or 30%, but will probably decline with improved understanding of the disease.[21,45] Many of the deaths are due to shock, which is seen during 10 to 50% of operations when the veins draining the tumor are ligated. This is believed due to the sudden deprivation of the large amount of pressor amines to which the organism has adapted. Disuse atrophy of the other adrenal medulla and surgical injury of the adrenal cortex may be contributing factors.[9,12]

Spontaneous shock has also been described. Whether this is due to sudden cessation

*Benzodioxane and dibenamine have also been employed to control the blood pressure during surgery.[45]

of secretion or is related to the shock-like state seen in epinephrine-infused animals is uncertain. Several reports describing such crises are in the literature. In some patients, deep shock alternates with bouts of severe hypertension.[9,11,42] Rarely shock appears as the presenting difficulty.[19] One report describes the appearance of shock following intramuscular administration of 10 mgs. of prochlorperazine.[2] The tumor may be infarcted and become functionless during the hypotensive episode.

Other complications include loss of vision, congestive failure, myocardial infarction, basilar thrombosis, severe diabetes, and, of course, the usual sequelae of hypertension.[20,26,50] Rarely, Addison's disease may result from compression of the adrenal cortex.[17,39] As already implied, when significant levels of pressor amines have been present long enough, hypertension may no longer depend on them, and may persist after otherwise successful surgery.[49]

SUMMARY

The diagnostic and therapeutic problems associated with pheochromocytoma are presented in detail. It is hoped that this will encourage increased recognition and more successful management of these tumors.

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REFERENCES

1. Bennett, M., and Walker, M.: Phaechromocytoma in pregnancy, Lancet 1:811, 1959.
2. Brody, I. A.: Shock after administration of prochlorperazine in patient with pheochromocytoma: report of a case with spontaneous tumor destruction, J.A.M.A. 169:1749, 1959.
3. Calvy, G. L., Resnick, M. E., Knab, D. R., and Richardson, J. F.: Hypertension, pregnancy, and pheochromocytoma, J.A.M.A. 171:151, 1959.
4. Colcock, B. P., and Hull, J. E.: Pheochromocytoma as a massive abdomnial tumor, J. Indiana M.A. 48:955, 1955.
5. Cook, J. E., Urich, R. W., Sample, H. G., Jr., and Fawcette, N. W.: Peculiar familial and malignant pheochromocytomas of the organs of Zuckerkandl, Ann. Int. Med. 52:126, 1960.
6. Corcoran, A. C., Dustan, H. P., and Page, I. H.: Antidiuresis and other renal responsesto piperoxan in pheochromocytoma, Circulation 14:221, 1956.
7. Davies, J. V. S. A.: Phaechromocytoma simulating thyrotoxicosis, Brit. M.J. 2:77, 1952.
8. Desai, P. B.: Microscopic pheochromocytoma of the adrenal, Indian J. Surg. 21:1, 1959.
9. Dybkoer, R. and Gjøl, N.: Pheochromocytoma: some symptoms and their patho-physiology, Acta Endocrinol. 26:450, 1957.
10. Emanuel, D. A., Rowe, G. G., Masser, M. J., and Philpot, Van B., Jr.: Prolonged hypotension with fatal termination after a phentolamine (Regitine) methanesulfonate test, J.A.M.A. 161:436, 1956.
11. Engel, F. L., Mencker, W. H., and Engel, G. L.: "Epinephrine shock" as a manifestation of pheochromocytoma of the adrenal medulla: report of a case with successful removal of the tumor, Am. J.M. Sci. 204:649, 1947.
12. Evans, V. L.: Suprarnal tumor with paroxysmal hypertension, J. Lab. Clin. Med. 22:1117, 1937.
13. Fränkel, F.: Ein fall von doppelseitigem, vollig latent verlaufenen Nebenierentumor und gleichzeitiger Nephritis mit Veranderungen am Circulationsapparat und Retinitis, Virchows Arch. F. path. Anat. 103:244, 1886.
14. Gilliland, I. C., and Daniels, O.: Phaeochromocytoma presenting as an abdominal emergency, Brit. M. J. 2:275, 1951.
15. Graham, J. P.: Pheochromocytoma and hypertension: an analysis of 207 cases, Internat. Abstr. Surg. 92:105, 1951.
16. Hingerty, D.: Thirty-minute screening test for phaeochromocytoma, Lancet 1:766, 1957.
17. Horn, H. L.: Pheochromocytoma: a case of paroxysmal hypertensive attacks induced by eating and change of position, Ann. Int. Med. 51:129, 1959.
18. Hutchison, G. B., Evans, J. A., and Davidson, D. C.: Pitfalls in the diagnosis of pheochromocytoma, Ann. Int. Med. 48:300, 1958.

19. Jelliffe, R. S.: Phaeochromocytoma presenting as a cardiac and abdominal catastrophe, Brit. M. J. 2:76, 1952.

20. Kvale, W. F., Roth, G. M., Manger, W. M., and Priestley, J. T.: Pheochromocytoma, Circulation 14:622, 1956.

21. Kvale, W. F., Roth, G. M., Manger, W. M., and Priestley, J. T.: Present-day diagnosis and treatment of pheochromocytoma: a review of fifty-one cases, J.A.M.A. 164:854, 1957.

22. Labbé, M., Tinel, and Doumer: Crises solaires et hypertension paroxystique enrapport avec une tumor surrenals, Bull. et mém. Soc. Med. d. hôp. de Paris 46:982, 1922.

23. Lamb, B. R. B., and Gresham, G. A.: Phaeochromocytoma of the urinary bladder, Lancet 1:81, 1958.

24. Lance, E. M., Cate, W. R., Jr., Liddle, G. W., and Scott, H. W., Jr.: Clinical experiences with pheochromocytoma, Surg. Gynec. & Obst. 106:25, 1958.

25. Lehman, D. J., Jr., and Rosof, J.: Massive hemorrhage into an adrenal pheochromocytoma: report of a case with sudden death, New England J. Med. 254:474, 1956.

26. Leiser, A. E., and Corcoran, A. C.: Antidiuretic response to piperoxan as a diagnostic test of pheochromocytoma, J.A.M.A. 162:540, 1956.

27. Lima, F. P., Barros, N. M., Jr., and Franca, H. H.: Pheochromocytoma: study of diagnostic tests: report of a case, Rev. Paulista Med. 50:111, 1957.

28. Litchfield, J. W., and Peart, W. S.: Phaeochromocytoma with normal excretion of adrenaline and noradrenaline, Lancet 2:1283, 1956.

29. Maier, H. C., and Humphreys, J., II: Intrathoracic pheochromocytoma: including a case of multiple paragangliomas of the functional and non-functional type, J. Thoracic Surg. 36:625, 1958.

30. Malbin, B.: Excess urinary catechol amines without pheochromocytoma, Ann. Int. Med. 51:613, 1959.

31. Masson, G. M. C., Corcoran, A. C., and Humphrey, D. C.: Diagnostic procedures for pheochromocytoma: technique of assay of urine in the rat and demonstration of piperoxan hydrochloride antidiuresis during normatensive phase, J.A.M.A. 165:1555, 1957.

32. Mayo, C. H.: Paroxysmal hypertension with tumor of retroperitoneal nerve, J.A.M.A. 89:1047, 1927.

33. Molinatti, G. M., Camanni, F., and Pizzini, A.: Malignant hypertension in a case of nonchromaffin paraganglioma with a high concentration of catecholamine, Cancer 12:878, 1959.

34. Moore, C., and Shumacker, H. B.: Adrenalin producing tumors in childhood, Ann. Surg. 143:256, 1956.

35. Orgain, E. S.: Pheochromocytoma: the value of certain tests used routinely in diagnosis, Ann. Int. Med. 43:1178, 1955.

36. Pincoffs, M. C.: A case of paroxysmal hypertension associated with suprarenal tumor, Tr. Assoc. Am. Physician 44:295, 1929.

37. Roland, C. B.: Pheochromocytoma in pregnancy: report of a fatal reaction to phentolamine (Regitine) methanesulfonate, J.A.M.A. 171:1806, 1959.

38. Rosenberg, L. M.: Pheochromocytoma of the urinary bladder: report of a case, New England J. Med. 257:1212, 1957.

39. Russel, D. S., Rubinstein, L. J., and Lumsden, C. E.: Pathology of tumours of the nervous system, ed. 1, London, Arnold, 1959, pp. 264-271.

40. Sandler, M., and Ruthvin, C. R. J.: Quantitative colorimetric method for estimation of 3-methoxy-4 hydroxymandelic acid in urine: value in diagnosis of phaeochromocytoma, Lancet 2:114, 1959.

41. Smithwick, R. H., Greer, W. E. R., Robertson, C. W., and Wilkins, R. W.: Pheochromocytoma: a discussion of symptoms, signs, and procedures of diagnostic value, New England J. Med. 242:252, 1950.

42. Terry, R. B., Tobin, J. R., and O'Connor, R. B.: Intravenous phentolamine for phaeochromocytoma and "adrenaline shock," Brit, M. J., 2:771, 1958.

43. Thorne, G. W., Nelson, D. H., and Renold, A. E.: Current status of the treatment of adrenal disorders, J.A.M.A. 168:2130, 1958.

44. Vaquez, H., and Donzelot, E.: Les crises d'hypertension arterielle paroxystique, Presse méd., 34:1329, 1926.

45. Von Euler, U. S., and Strom, G.: Present status of diagnosis and treatment of pheochromocytoma, Circulation 15:5, 1957.

46. Weil-Malherbe, H.: Phaeochromocytoma: catechols in urine and tumor tissue, Lancet 2:282, 1956.

47. Weinberg, J.: In, Questions and answers, J.A.M.A., 168:2208, 1958.

48. Wingo, C. F., Williams, J. P., and Wade, F. A.: Pheochromocytoma: two case reports with unusual reactions and a general review, Ann. Int. Med. 42:856, 1955.

49. The diagnosis of pheochromocytoma (editorial), Ann. Int. Med. 39:1333, 1953.

50. Pheochromocytoma (leading article), Lancet 1:1080, 1959.

51. Kelly, A.: Personal communication, 1960.

A REVISED SUTURE METHOD FOR REPAIR OF HEPATIC LACERATIONS

An Experimental Study with an Illustrative, Clinical Case

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Past experience with the conventional, operative techniques for the emergency repair of traumatic, hepatic lacerations has proved less than satisfactory in our hands. Some of the various methods that have been advocated by surgeons during the past fifty years are listed in Table I. Most of these techniques employ specific,

Table I

HEPATIC SUTURE METHOD

TYPES OF SURGICAL PROCEDURES

1. Suturing — simple and special hemostatic sutures
2. Suturing of hemostatic agents into wound
3. Suturing of hemostatic packs into wound
4. Free surgical packs — secondary removal
5. Surface coverage with S - T skin autographs
6. Surface coverage with free peritoneal autografts
7. Debridement

hemostatic agents to aid in the control of bleeding, and it is usually recommended that such agents be placed in the lacerations, between the raw surfaces, at the "fracture" site. Various types of agents have been advocated, including commercial hemostatic agents such as Gelfoam or Oxycel, autogenous muscle, and autogenous split-thickness skin grafts.[1,6] An undesirable effect has been observed with the placement of the more bulky of these substances on the raw surfaces of the liver in the crevice of a hepatic laceration.

In order to evaluate this concept, the problem was taken to the experimental laboratory where two series of dogs were prepared. Two different types of repairs were carried out on experimentally produced hepatic lacerations.

METHOD

Ten dogs were aseptically operated upon, under nembutal anesthesia, through a midline, epigastric incision. A one and one-half to two inch, linear, full thickness laceration of either the central or left lateral lobe of the dog's liver was produced with a piece of rope silk used in a sawing manner. Brisk arterial and venous bleeding occurred in most instances. The presence of gross bile duct damage was not as easily determined.

Two types of repairs were then employed with this standard preparation, the two types being alternated, thereby producing two series of five each, Table II.

Table II

HEPATIC SUTURE METHOD

EXPERIMENTAL DATA*

	Group A (Buried)		Group B (Surface)	
Survived	3	60%	5	100%
Died	2	40%	0	0%

*Observation period — 5 to 14 days

Group A, the standard or conventional type of repair served as a control. In this

*Division of General Surgery.

group, obvious bleeding points were suture ligated and then Gelfoam was placed on the raw or cut surfaces of the laceration and the surfaces were then approximated with heavy, through and through sutures of 2-0 braided, black silk.

In Group B, the surfaces were inspected and all bleeding points were suture ligated with fine, swedged on arterial silk on tapered, round needles. Gauze sponge pressure was held on the raw surfaces for several minutes and then the raw surfaces were approximated and sutured together with superficial, figure-of-eight sutures placed into Glisson's capsule just adjacent to the wound margin. In this manner the defect was filled with a thin layer of fibrin but no foreign matter entered the "fracture" site. Oxycel cotton was used in this series but it was not placed into the gap between the raw surfaces but rather, it was placed in the free loops of the figure-of-eight sutures so as to seal the crevice on the surface of the liver. The Oxycel cotton was rolled into small, compact cylinders and in this form it easily tied down under the figure-of-eight sutures.

Both groups were followed for periods of five to fourteen days and then sacrificed and autopsied to determine the status of the repair. Two dogs in Group A died within 72 hours and they were autopsied. None of the animals were given blood transfusions, intravenous infusions, or antibiotics either during operation or post-operatively. None of the animals had their abdominal cavities drained.

RESULTS

In Group A, a mortality of forty percent was observed. This came as a result of two dogs dying within 72 hours of bile peritonitis. The findings were classical at autopsy and in neither animal had there been gross evidence of damage to the intrahepatic, bile duct system at the time of trauma or repair.

In both Group A and B, there was no evidence of significant postoperative hemorrhage, nor could a significant difference be ascribed to the specific type of hemostatic agent employed.

Figure 1 shows a specimen from a dog in Group B and it is graded as an excellent result. A smooth, healthy, shiny layer of epithelium has covered the original, raw surface of the liver laceration. This appears to be a contribution of the omentum to the healing process. No hemostatic material was placed into the laceration but rather on the approximated, liver wound margin.

Figure 2 represents a specimen from Group A in which the repair was carried out with buried Gelfoam. Two recovered specimens in this group showed this type of reaction with the buried, hemostatic material. When opened, these intrahepatic areas were cystic and contained serum and necrotic liver tissue. It should be noted that this particular site was adjacent to, but not in direct communication with the bed of the gallbladder.

DISCUSSION

In this small series of two groups of five animals each, great significance can not be placed on the comparison of the mortality rates of the two methods. The forty percent mortality with buried hemostatic material was related to bile peritonitis

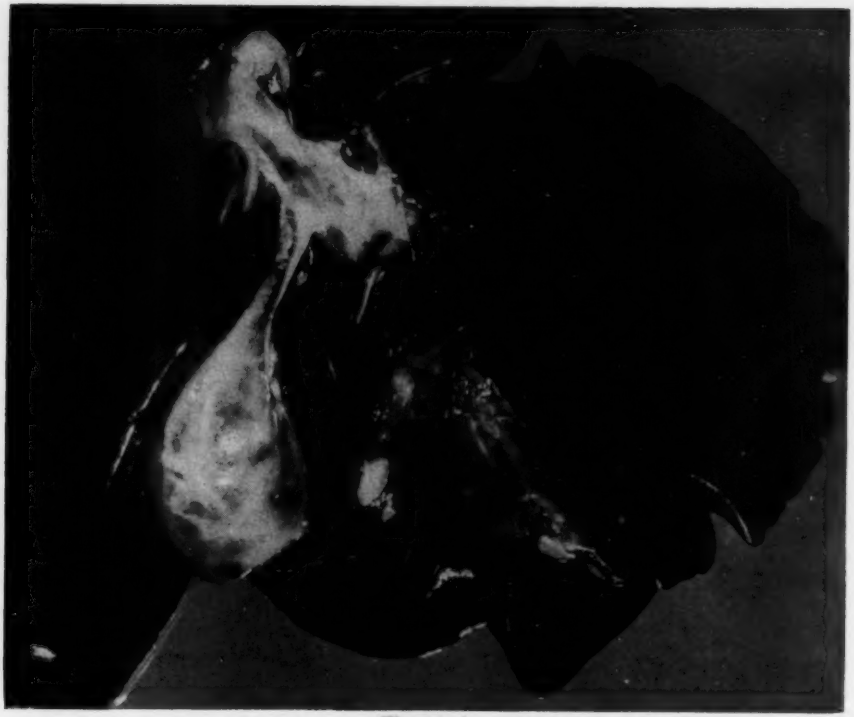

Figure 1

The experimentally produced laceration in the left lobe of the liver shows excellent healing at the "fracture" site. The fibrous and foreign body reaction has been minimal since no suture material or hemostatic agent was wedged into the "fracture" site.

which is a lethal condition in the dog. The same set of circumstances in a human case, well drained, might have only resulted in added morbidity.

Certain, more subtle changes were noted in the area of repair at the time of sacrifice and autopsy. The burying of hemostatic material in the substance of the liver, while attempting to close the fracture, produces two unfavorable effects. First it serves as a mechanical wedge, much like the steel wedge the woodcutter uses to split a log. Figure 3 depicts the way in which buried hemostatic material can work against the surgeon and make the fracture more pronounced. Nothing is superior to the apposition of the two matching, raw surfaces of the liver, each covered with a thin coating of fibrin. Careful suture ligation of gross vessels and ducts that have been damaged is essential and is most satisfactorily done with arterial silk on an atraumatic needle. Secondly, the buried hemostatic agents are difficult to place so as to get all of the raw surfaces covered and held with pressure to prevent the leakage of bile from the smaller ducts that would not be visualized and controlled by direct, suture ligation. The natural and exact fit obtained when the two raw surfaces are placed face to face is excellent; buried, foreign material would spoil this approximation.

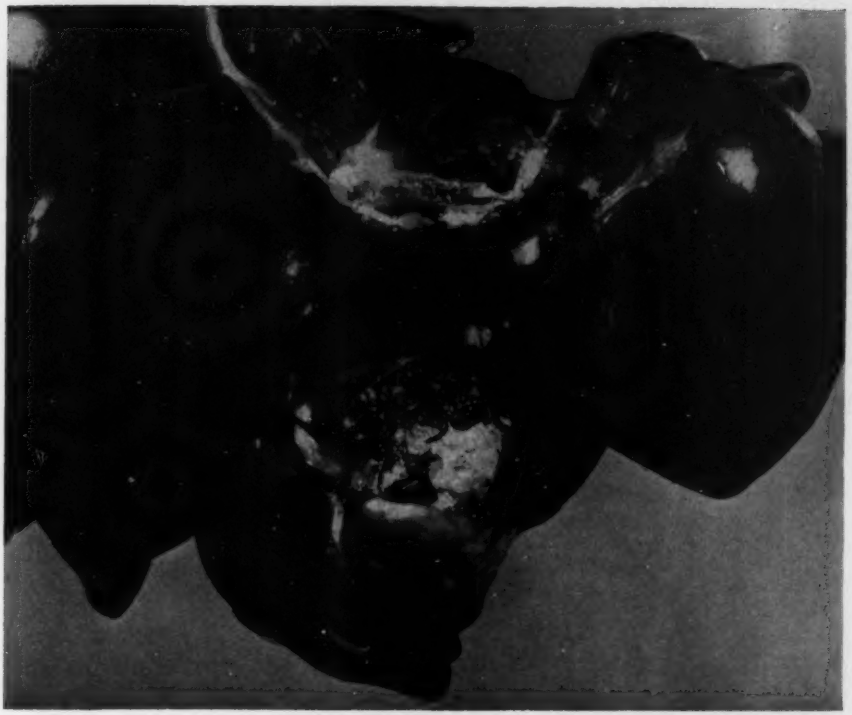

Figure 2

The reaction of the liver at the "fracture" site in this dog from group A has been excessive and disorderly with areas of hepatic necrosis, a consequence of pressure and ischemia caused by the wedged in hemostatic agent and the deep sutures.

A gentle, non-strangulating, figure-of-eight suture placed to hold Glisson's capsule at the liver wound margin with the addition of Oxycel cotton pledgets placed under the free loop of the suture so as to cover the wound's free margin, gives the finest repair of a hepatic laceration in our hands.

Early in the autopsy study, it was noticed that the omentum appeared to contribute much in a favorable way to the best repairs. The smooth, glistening surface seen on the inner surfaces of the fracture shown in Figure 1, is thought to be a contribution of the omentum. Buried hemostatic material hindered this ingrowth in Group A. Placement of the free omentum over the operative site at the completion of the repair is considered advisable.

A clinical case of traumatic, hepatic fracture entered the Henry Ford Hospital via the emergency room and has given us an opportunity to put these concepts into practice.

T. W., a 64 year old male was lying on his back, working under his automobile on 4-28-57 when it fell off its jacks and crushed the patient, pinning him to the

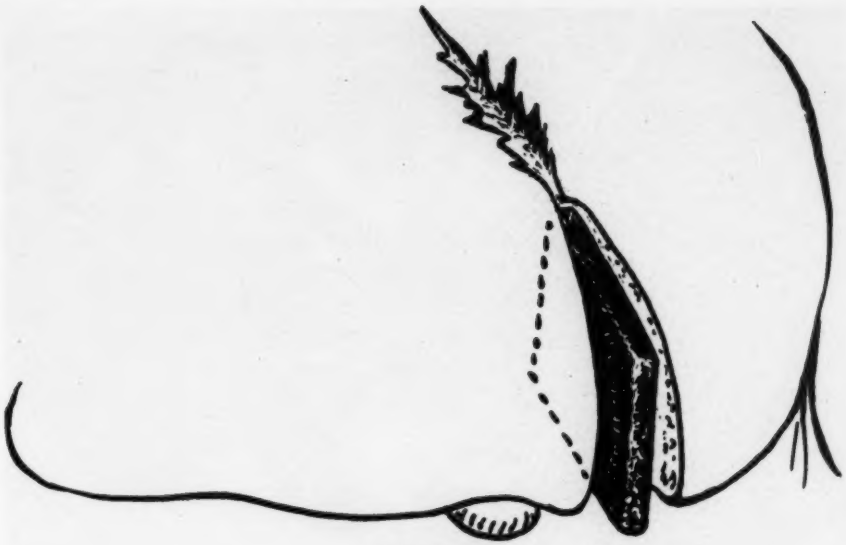

Figure 3

Hemostatic agents wedged into the "fracture" site extends the fracture as does the woodcutter's wedge.

ground. The patient was brought to the emergency room shortly after being removed from under the automobile by the police. On admission several finding were prominent; the patient's blood pressure was 90/60 mm. of Hg., there was a left, posterior 4th rib fracture, and there was muscle guarding in the right upper quadrant of the abdomen with moderate, upper abdominal distention. An upper abdominal, diagnostic paracentesis was positive with bright red blood, which showed no tendency to clot.

Preparations were made for laporatomy with a preoperative diagnosis of ruptured spleen and, or liver. The preoperative hemoglobin was 12.4 grams per 100 ml. The first of three units of blood was begun as the patient was intubated for anesthesia. A midline, epigastric incision was made to enter the abdomen where free and clotted blood was encountered. Exploration revealed the spleen to be normal and the liver was found to be the source of the hemorrhage. It was then seen that the exposure via this incision would be inadequate for the repair of a large, stellate fracture of the right lobe of the liver and it was necessary to extend the incision through the diaphragm into the right hemithorax via the eighth interspace, thereby obtaining adequate exposure of the entire right lobe of the liver.

Fine silk, suture ligatures were placed on all grossly visable, damaged vessels and bile ducts. Larger, curved, fine diameter, round needles carrying 2-0 black silk were used as figure-of-eight sutures to approximate the fracture lines. As these sutures were tied, cylindrical wisps of Oxycel cotton were placed under the free loops so that the Oxycel drew down *onto* and not into the fracture sites. A very satisfactory and dry repair was obtained and the operative incision was then closed in layers. Both the abdominal and right thoracic cavities were drained. The post-

operative course was complicated by phlebothrombosis with pulmonary embolism, and an accumulation of bile and necrotic hepatic tissue in the right upper quadrant. The phlebothrombosis and embolism prompted the performance of a bilateral, superficial femoral vein ligation and the right upper quadrant accumulation drained spontaneously through one of the old drain sites. The patient was discharged from the hospital two months postoperatively. At the time of discharge, the liver function tests had returned to normal as shown in Table III, and all of the wounds were healed. The patient has just recently been seen in the outpatient clinic and his general health continues to be excellent for a man his age.

Table III

HEPATIC SUTURE METHOD

Laboratory Data					Clinical Case		T. W.	
Date	C. C.	T. T.	T. F.	P. T.	A./G. ratio	B. S. P.	Cholesterol	Transaminase
4-28-57	Operation			87%				
5-1 6-57	1+	1u	0	63%		30%	134 esters 55%	24
5-29-57	1+	3u	1+	67%	4.0/3.7	13%	242 esters 46%	28
6-27-57	0	3u	0	87%	4.6/3.1	7%	326 esters 79%	20

CONCLUSIONS

When the diagnosis of hepatic laceration or fracture has been made and the patient has been transported to the operating room for surgical repair, the following points are offered as basic factors in a suture method for hepatic laceration repair:

1. Adequate exposure of the entire liver must be obtained and for major hepatic injuries, this usually requires a thoraco-abdominal incision. The initial incision for laporatomy in cases of suspected liver injury are thus placed so as to allow easy conversion to such a thoraco-abdominal incision.

2. Gross blood vessels and bile ducts seen on the surfaces of the hepatic injury should be suture ligated under direct vision.

3. Suture approximation of the hepatic wounds with sutures placed to avoid strangulation of large portions of liver parenchyma and utilizing Glisson's capsule is desirable. Hemostatic material used in conjunction with such a suture should be placed on the wound margin and not down into the depth of a wound.

4. The omentum should be placed in the upper abdomen, over the fracture sites, at the completion of the procedure so that it may give its fullest benefit to the reparative process.

5. Adequate, prolonged, drainage of the operative area is advised.

Following the repair of a hepatic laceration, a stormy postoperative course should be anticipated, and then the benefits of meticulous care, both operative and postoperative, will be rewarding to both the patient and the surgeon.

REFERENCES

1. Bailey, O. T., Ingraham, F. D., Swenson, O., Lowrey, J.J., and Bering, E. A., Jr.: Human fibrin foam with thrombin as a hemostatic agent in general surgery, Surgery 18:347, 1945.

2. Frantz, V. K., Clark, H. T., and Lattes, R.: Hemostasis with absorbable gauze (oxidised cellulose) Ann. Surg. 120:181, 1944.

3. Horton, C., Georgiade, N., Campbell, F., Masters, and Pickrell, K.: Behavior of split thickness and dermal skin grafts in peritoneal cavity; experimental study, Plast. & Reconstruct. Surg. 12:269, 1953.

4. Jones, T. W., Nyhus, L. M., and Harkins, H. N.: Formalinized polyvinyl alcohol (Ivalon) sponge in repair of liver wounds, A.M.A. Arch. Surg. 76:583, 1958.

5. Jenkins, H. P.: Control of hemorrhage by gelatin sponge, J. Internat. Coll. Surgeons 10:521, 1947.

6. Masters, F., Georgiade, N., Horton, C., and Pickrell, K.: Control of liver hemorrhage by split-thickness skin grafting; experimental study, A.M.A. Arch. Surg. 69:718, 1954.

CHRONIC PULMONARY DISEASES AND ASSOCIATED NEUROLOGIC DISTURBANCES*

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In recent years, renewed interest in the neurological manifestations and complications of chronic pulmonary diseases has been aroused. Cameron in reporting the case of a young coalminer with pulmonary emphysema, visual disturbances, and papilledema, appears to have been the first to emphasize the association between these seemingly disparate disorders.[1] More recently, Austen has suggested a clinical syndrome consisting of signs and symptoms of pulmonary and cardiac failure, headache, papilledema, involuntary movements of the extremities, and impairment in the level of consciousness.[2] Chronic pulmonary insufficiency with its resultant hypoxia and hypercapnia appears to be the direct or indrect influencing factor in the production of these symptoms.

The primary problem of pulmonary insufficiency is common, and the neurologic complications relatively uncommon. The importance of recognizing this association is imperative if unnecessary diagnostic or surgical procedures are to be avoided. Frequently, the neurologic symptoms are so predominant that they may obscure the underlying pulmonary disease. With specific regard to this point Conn reported two cases in which the diagnosis of brain tumor was mistakenly made and in which the degree of the associated pulmonary disease was unrecognized.[3] The true nature of the syndrome was revealed by necropsy in both cases. No evidence of an intracranial lesion was found and only signs of increased intracranial pressure were observed.

Also important in the recognition of the above syndrome is the necessity of instituting corrective therapy and, more specifically, that the therapy be directed at the primary underlying disorder. In most instances, measures that have corrected the hypoxia, hypercapnia, cardiac failure, and polycythemia, have resulted in complete remission of neurologic symptoms.

The usual clinical picture observed is that of a middle-aged individual with a longstanding history of chronic asthma, bronchitis, and empysema, and with progressive development of pulmonary and cardiac failure, headaches, visual disturbances, papilledema, tremor and twitching of the extremities, and impairment of consciousness.

Chronic pulmonary insufficiency is the underlying disorder, but the specific pulmonary disease frequently differs in each case. Chronic bronchitis and asthma are by far the most common etiologic disorders. In none of the reported cases has the subsequent correct diagnosis proved to be brain tumor, either clinically or with post-mortem examination, as in three instances cited by Conn.[3] Nevertheless, the differential diagnosis remains difficult, especially in those cases in which signs of increased intracranial pressure persist despite adequate corrective therapy. In these situations, exhaustive efforts must be made to exclude the presence of an intracranial space-occupying lesion.

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Congestive heart failure is present in almost every case. Both sides of the heart may be affected, although right ventricular decompensation is most common and manifested variously by elevated venous pressure, hepatomegaly, peripheral edema, and usually, EKG findings of right axial deviation and right ventricular hypertrophy. Left heart failure is generally indicated by the presence of dyspnea, basal rales, and pulmonary congestion.

Headache is an early and common neurologic symptom occurring in approximately fifty percent of the cases.[2] It is quite variable in location and may be generalized. It is frequently severe and intense, persistent and steady. Often the headache is nocturnal or occurs in the early morning hours, sometimes awakening the patient from a sound sleep. Nausea and vomiting are at times related to the headaches but are not a frequent feature of the syndrome.

The exact mechanism of headaches in these cases is not clearly established but is probably related to the carbon dioxide retention and resultant cerebral vasodilatation and increased cerebral blood flow, rather than to increased intracranial pressure.[2,3]

Papilledema is a distinctive feature of the syndrome but cannot be distinguished from other causes of this symptom. It may be unilateral or bilateral, and varies in severity from haziness or blurring of the disc margins, to markedly choking, venous congestion, and retinal hemorrhages. Visual acuity may be significantly impaired but usually is not altered. Papilledema has been reversible in nine cases reported, following improvement of the cardiopulmonary status. The relationship between the severity of the papilledema and elevated cerebrospinal fluid pressure is unpredictable.

Manifestations of cerebral dysfunction vary widely and may include drowsiness and hypersomnolence, or even stupor and coma. The patients are at times forgetful, inattentive, irritable, and easily confused. Often they respond sluggishly to verbal and noxious stimuli and may appear disoriented and incoherent. Evidence of specific cerebral deficits, such as motor and sensory paralysis, aphasia, and visual—spacial disorientation, is usually lacking.

Tremor and twitching of the extremities are conspicuous and characteristic findings in the more recently reported cases.[2] The tremor is typically of the action type, demonstrated in the outstretched fingers, persisting throughout voluntary movement, and aggravated slightly with effort. The coarse twitching movement of the extremities is reported identical to that seen in hepatic coma, and is arrhythmic and asynchronous. It is usually demonstrated with the arms outstretched and the fingers and wrist in dorsiflexion. Similar twitching of the legs, eyelids, and mouth, are seen following activation and contraction of the respective muscles.

The exact mechanism of the movement disorder is unknown, but hypercapnia, ammonia intoxication, and acidosis, have been suggested as possible factors in its production. Carbon dioxide retention as a possible causative factor has also been suggested because of the observation of myoclonic movements and coma occurring in some patients with pulmonary insufficiency receiving oxygen therapy.[4,5] These

signs usually disappeared when the oxygen was discontinued. Furthermore, rhythmic movements of the extremities have been described in patients receiving carbon dioxide therapy.[6]

Blood ammonia determinations were performed by Austen because of the striking resemblance of the twitching movements to that seen in hepatic coma.[2] The results were variable, and ammonia intoxication was not definitely established as a cause for this sign. Liver function tests were generally negative, and ammonium chloride administration after the twitching subsided did not necessarily produce its recurrence.

It has been suggested that increased acidosis may account for the twitching. However, this possibility seems less likely since twitching occurs in some patients with pulmonary insufficiency in the absence of acidosis, and in hepatic coma in which alkalosis reportedly occurs. Furthermore, in diabetic acidosis twitching has not been observed.[2]

The EEG findings are non-specific and consist primarily of increased voltage and a diffuse slowing of the electrical activity in the delta and theta frequencies. However, these findings occur in all types of coma due to metabolic disturbances and are frequently reversible.

In general, the various neurologic manifestations are in some way related to the underlying cardiopulmonary insufficiency with its resultant hypoxia and hypercapnia. However, the mechanisms for the production of the individual disturbances is variable and uncertain.

The occurrence of papilledema has been reported in twenty-five cases of chronic pulmonary insufficiency. The probable factors contributing to the production of this important sign include, 1) increased venous pressure, 2) hypoxia and hypercapnia, and 3) polycythemia.

Elevated venous pressure is known to produce increased intracranial pressure as manifested by the performance of the Queckenstedt maneuver. However, papilledema and other symptoms and signs of increased intracranial pressure are not necessarily produced by increased venous pressure.[7,8] Evidence to this effect is the absence of papilledema in cases of right heart failure, and in mediastinal tumors producing obstruction of the superior vena cava, despite high venous pressures exceeding four hundred millimeters of water. Furthermore, in the majority of cases of chronic pulmonary insufficiency and papilledema, the venous pressure has been found to be normal or mildly elevated.[9,10]

Beaumont reported a case of papilledema associated with heart failure and attributed the papilledema to increased pressure in the cranial sinuses resulting in interference of filtration of the arachnoid villi, and a rise of cerebrospinal fluid pressure.[11]

Secondary polycythemia may occur as a physiologic response to chronic anoxia in chronic pulmonary disease. An increase in the concentration of formed elements in the blood may occur, changing the dynamics of the intracranial venous pressure

and resulting in increased blood viscosity. This has been suggested as the possible mechanism for the production of papilledema by several investigators.[12,13] However, it is unlikely that polycythemia is a major factor in the production of papilledema, a view supported by Simpson's observation of only one case of secondary polycythemia in eleven patients with pulmonary insufficiency and papilledema.[9,10] Furthermore, uncomplicated primary polycythemia is rarely associated with papilledema.[2,14] Nevertheless, the association of papilledema and polycythemia is an important one and should raise the possibility of an intracranial expanding lesion. An example of this is hemangioblastoma of the cerebellum associated with polycythemia.

Another contributing factor in the development of papilledema is congestive heart failure as proposed by Austen because of its occurrence in nearly all of the twenty-five cases reported.[2] The pulmonary congestion is thought to augment blood-gas abnormalities, resulting in papilledema.

The most likely cause for the production of papilledema seen in pulmonary insufficiency appears to be the presence of hypoxia and hypercapnia, which are known to produce edema and congestion of the brain. This has been suggested by the post-mortem findings of cerebral edema and congestion in thirty-six cases of pulmonary insufficiency reported by Simpson.[10] Our examination of necropsies of cases with chronic pulmonary insufficiency has also confirmed the presence of marked congestion and edema of the brain manifested by flattening of the cerebral convolutions, decrease in the size and sometimes near-collapse of the ventricles, and occasional pressure cone due to herniation of the temporal lobes or cerebellum.

Both an elevated carbon dioxide content and a low oxygen saturation may result in cerebral vasodilatation, increasing the cerebral blood flow, producing elevation of the cerebrospinal fluid pressure[15] and papilledema.[4,10] The effect of carbon dioxide tension is thought to be greater than that of hypoxia in producing these changes.

One problem which remains unanswered, however, is the development of papilledema in some cases of cardiopulmonary insufficiency and not in others. To add further confusion, a case of papilledema and diffuse emphysema, in the absence of hypercapnia, has been recently reported by Leggat.[16] This stresses the need for further evaluation of this problem which is made difficult because treatment is necessarily directed at all the symptoms simultaneously, not allowing individual investigation of each contributing factor.

The mental changes that accompany chronic pulmonary insufficiency have been attributed to elevated carbon dioxide tension. In this regard certain observations are of importance.

Carbon dioxide retention is known to cause increased intracranial pressure, presumably as a result of cerebral vasodilatation and subsequent increased cerebral blood flow.[4,9,17] Furthermore, it has been suggested that a fall in arterial pH accompanying hypercapnia may produce a diminished cerebral oxygen consumption by interfering with its utilization.[18]

The explanation of McCann ascribed to the development of cerebral symptoms in chronic pulmonary insufficiency, is that of impaired circulation at the capillary level due to increased alveolar pressure, as well as diminished negativity of the intrapleural pressure.[19] Further impairment of venous return and insufficient cardiac output may occur due to lack of compensatory rise in intra-abdominal pressure.

More confirmatory evidence suggesting the probable role of hypercapnia in the development of alterations of consciousness, has been the observation of mental changes occurring in anoxemic patients following the administration of oxygen, especially in high concentration.[19,5,10] These changes range from somnolence and mild confusion, to coma and even death. Parodoxically, alleviation of these symptoms followed almost immediately upon discontinuation of the oxygen. The explanation for this phenomenon is that during chronic elevation of the carbon dioxide tension, the respiratory center is no longer responsive to changes in blood carbon dioxide levels. Hypoxia then takes over as the major respiratory stimulus through its reflex action on the chemoreceptors of the carotid and aortic-bodies. When oxygen is administered, this effect is abolished resulting in hypoventilation, further carbon dioxide retention, and subsequently coma.[3,20] Such situations may usually be prevented with the use of mechanical body-respirators producing hyperventilation and reversing the elevated carbon dioxide content without significantly altering respiration during oxygen therapy. In addition, gradually increased concentrations of oxygen have been recommended.[21] It is important to recognize the actual cause of impaired consciousness in these cases, especially where oxygen is used, since the sudden progression of symptoms may be wrongly attributed to the disease, rather than to its therapy.

The purpose of the above presentation has been to stress the existence of a few of the more common neurologic features of chronic pulmonary disorders in the hope that early recognition will avoid misdirected therapy and unnecessary surgical procedures.

REFERENCES

1. Cameron, A. J.: Marked papilloedema in pulmonary emphysema, Brit. J. Ophth. 17:167, 1933.

2. Austen, F. K., Carmichael, M. W., and Adams, R. D.: Neurologic manifestations of chronic pulmonary insufficiency, New England J. Med. 257:579, 1957.

3. Conn, H. O., Dunn, J. P., Newman, H. A., and Belkin, G. A.: Pulmonary emphysema simulating brain tumor, Am. J. Med. 22:524, 1957.

4. Davies, C. E., and MacKinnon, J.: Neurological effects of oxygen in chronic cor pulmonale, Lancet 2:883, 1949.

5. Comroe, J. H., Jr., Bahnson, E. R., and Coates, E. O., Jr.: Mental changes occurring in chronically anoxemic patients during oxygen therapy, J.A.M.A. 143:1044, 1950.

6. Medunn, L. J.: Carbon Dioxide Therapy, Springfield, Thomas, 1950.

7. Friedfeld, L., and Fishberg, A. M.: Relation of cerebrospinal and venous pressures in heart failure, J. Clin. Invest. 13:495, 1934.

8. Harrison, W. G., Jr.: Cerebrospinal fluid pressure and venous pressure in cardiac failure and effect of spinal drainage in treatment of cardiac decompensation, Arch. Int. Med. 53:782, 1934.

9. Simpson, T.: Papilloedema in emphysema, Brit. M. J. 2:639, 1948.

10. Simpson, T.: Acute respiratory infections in emphysema; account of 118 cases, Brit. M. J. 1:297, 1954.,

11. Beaumont, G. E., and Hearn, J. B.: Case of reversible papilloedema due to heart failure, Brit. M. J. 1:50, 1948.

12. Meadows, S. P.: Papilloedema associated with chronic bronchitis, emphysema and polycythaemia, Proc. Roy. Soc. Med. 40:555, 1947.

13. Loman, J., and Dameshek, W.: Plethora of intracranial venous circulation in case of polycythemia; pathologic physiology and diagnostic considerations, New England J. Med. 232:394, 1945.

14. Drew, J. H., and Grant, F. C.: Polycythemia as neurosurgical problem; review with reports of 2 cases, Arch. Neurol. & Psychiat. 54:25, 1945.

15. Patterson, J. L., Jr., Heyman, A., and Duke, T. W.: Cerebral circulation and metabolism in chronic pulmonary emphysema with observations on effects of inhalation of oxygen, Am. J. Med. 12:382, 1952.

16. Leggat, P. O.: Diffuse pulmonary emphysema and papilloedema, Lancet 1:672, 1958.

17. Wolff, H. G., and Lennox, W. G.: Cerebral circulation; effect on pial vessels of variations in oxygen and carbon dioxide content of blood, Arch. Neurol. & Psychiat. 23:1097, 1930.

18. Mithoefer, J. C.: Increased intracranial pressure in emphysema caused by oxygen inhalation, J.A.M.A. 149:1116, 1952.

19. McCann, W. S.: Effects of emphysema of lungs on cerebral and coronary circulation, Postgrad. Med. 9:225, 1951.

20. Carter, C. C., and Fuller, T. J.: Increased intracranial pressure in chronic lung disease, Neurology 7:169, 1957.

21. Barach, A. L.: Physiological methods in diagnosis and treatment of asthma and emphysema, Ann. Int. Med. 12:454, 1938.

THE RESPONSE OF SINUS NODE FUNCTION TO LIGATION OF THE SINUS NODE ARTERY*

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Experimental production of ischemia of the sinus (sinoatrial) node alone is extremely difficult. Halpern,[1] on the basis of careful anatomic studies of 107 canine hearts, concluded that occlusive vascular changes could not affect sinus node function because of extensive nutrient anastomoses.

In a recent publication Botti and his colleagues[2] claim to have produced consistent alterations in sinus node function by ligating the sinus node artery. We felt that such an important observation deserved further investigation.

METHOD

In 16 dogs, following intravenous administration of nembutal (30 mg./Kg.), an endotracheal tube was inserted and a right thoracotomy performed. The right

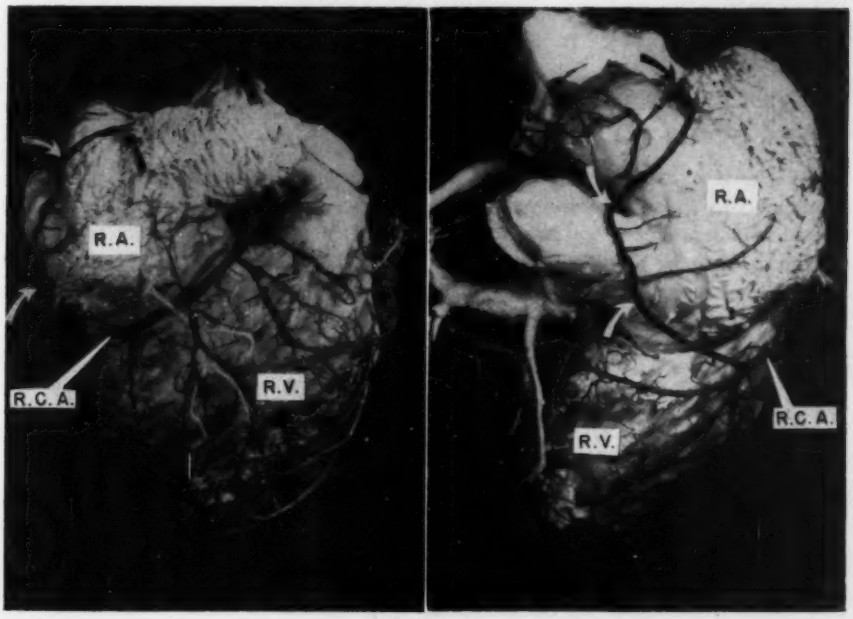

Figure 1

Vinylite cast of the coronary arteries and the right atrium (R.A.) and right ventricle (R.V.) of a dog, demonstrating the typical location of the artery supplying the sinus node. It is usually a terminal branch of the right coronary artery (R.C.A.). A is a view of the anterior aspect of the right chambers; B is a posterior view. In both views the white arrows indicate the sinus node artery; the single black arrow in each view indicates the location of the crista terminalis, seat of the sinus node. The crista terminalis is located between the right atrial appendage and the superior vena cava. In view B the cast of the superior vena cava is seen between the black and the upper white arrows.

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atrium was exposed and the artery supplying the crista terminalis identified and ligated (Figure 1); in 2 dogs there were two major arteries supplying this area and both were ligated. All six limb leads of the electrocardiogram (three bipolar and three augmented unipolar leads) were recorded before surgery. Of these six leads the one best showing P waves was selected for monitoring; in all animals this was either lead aVR or II. After ligation of the sinus node artery the electrocardiogram was recorded constantly for 5 minutes, then every minute for 10 minutes, every two minutes for 15 or 20 minutes, and approximately every 10 minutes for the remainder of an hour. Every dog was observed at least an hour with the exception of four, two of whom died prematurely due to technical errors and two others in whom tracings were obtained for only 30 minutes. This schedule of electrocardiographic recording was supplemented by direct observation of atrial activity throughout the experiment and additional records were made at any time abnormal activity was observed.

In 70-90% of dogs the sinus node artery arises from the distal portion of the right coronary artery.[1,3-6] In humans there is also only one major atrial artery supplying the sinus node, but arising from the *proximal* third of the right (60%) or left (40%) coronary artery.[7]

RESULTS

Table I and Figures 2 and 3 present the electrocardiographic changes encountered. In five of the sixteen dogs there were no changes following ligation of the sinus node artery. Two dogs showed minor changes, which were not considered significant. In nine of the sixteen dogs major P wave changes or arrhythmias, most of which were transient, developed as a result of the experiment. Two of the latter group of nine dogs developed ventricular ischemia because of blood loss (dog #10) and asphyxia (dog #5).

DISCUSSION

Our findings fail to confirm those reported by Botti's group that sinus node function can *regularly* be altered by occlusion of the sinus node artery. Most of the changes we observed following ligation of this artery were non-specific, of the type seen to occur spontaneously in a dog with heart exposed.

It is difficult to decide which electrocardiographic changes would be due specifically to ligation of the sinus node artery. Atrial premature beats, for example, may be a result either of the experimental procedure or may be unrelated, for they certainly occur both spontaneously and as a result of simple surgical exposure of the heart. Of the variety of changes which were observed, we feel the one which most dependably can be attributed to sinus node artery ligation is sinus arrest. This was observed only once, in dog number 10, where it occurred terminally during a period of hypotension and ventricular ischemia (Figure 3).

Minor changes in P wave voltage may have been a result of the artery ligation but could also occur with a number of other events, such as a change in the anatomic position of the heart in the opened chest. Gross variation in voltage, particularly when in abrupt sequence, was probably not due to position variation

Table I

Dog No.	Duration Observation	EKG Changes	Comments
1.	60 min.	Minor P wave changes, first 35 minutes.	Normal at 60 min.
2.	75 min.	Loss of P voltage at 30 sec.; combination (fused) beats at 45 sec.; loss of P voltage at 1 min. 45 sec. again; delta waves at 4 min.	Stable P waves after 4 min., for duration of experiment.
3.	75 min.	No changes.	
4.	120 min.	Occasional atrial prematures at 4 minutes.	SA node artery indistinct.
5.	15 min.	Atrial prematures at 2½ and 3 min.; gross P voltage variation at 7 min.; giant P waves and ventricular ischemia terminally (at 15 min.)	Accident with automatic respirator killed animal.
6.	30 min.	No Changes.	Two major atrial arteries, supplying sulcus terminalis; both ligated.
7.	33 min.	No Changes.	
8.	120 min.	Change in atrial vector immediately after ligation; at 3 min. atrial prematures plus nodal escape beats; atrial prematures at 9 min. with nodal escape rhythm; abrupt giant P wave at 30 min. (Fig. 2),	After 10 min., normal sinus rhythm rest of experiment.
9.	60 min.	Atrial prematures at 4 min.; shifting pacemaker at 60 min.	
10.	25 min.	Ventricular ischemia at 15 min.; at 22 min. sinus arrest, becoming intermittent at 25 min. (Fig. 3).	Coronary artery laceration with blood loss at 18 min., cause of death.
11.	60 min.	Q before P wave at 30 min.; atrial prematures at 60 min.	2 major atrial arteries from right coronary artery ligated.
12.	60 min.	Multiple nodal prematures at 5, 9, 14, 16 and 20 minutes.	After 20 min., sustained normal sinus rhythm.
13.	60 min.	No changes.	
14.	60 min.	Nodal and ventricular prematures at 30 sec.; change in P waves vector transiently at 10 min., nodal rhythm at 55 min.	
15.	60 min.	Atrial prematures at 45 sec.; P-Tp depression at 30 min. and 60 min., both tansiently.	
16.	60 min.	No changes.	

alone. The giant P waves occurring terminally in dog number 5, and associated with ventricular ischemia, are probable evidence of atrial injury, as was the P-Tp depression in dog number 15.

Difficulty in producing isolated sinus node ischemia is due not only to extensive collateral blood supply but also to the unimpaired efficiency of the left ventricle, the source of perfusion pressure for the two coronary artery beds. Ischemia produced

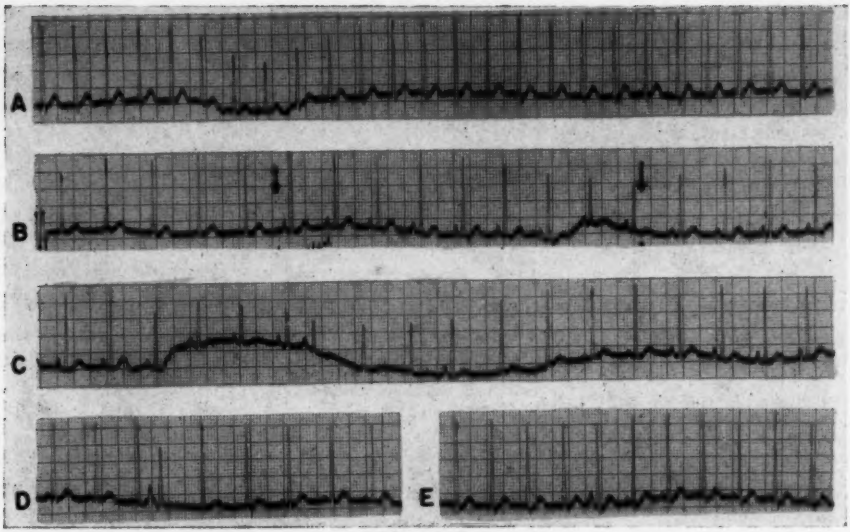

Figure 2

Electrocardiograms from Dog 8 (all lead II). A is a control tracing; B is at the time of ligation, begun at the first arrow and completed at the second; C is 3 minutes later, D 9 minutes and E 30 minutes. See Table I for description.

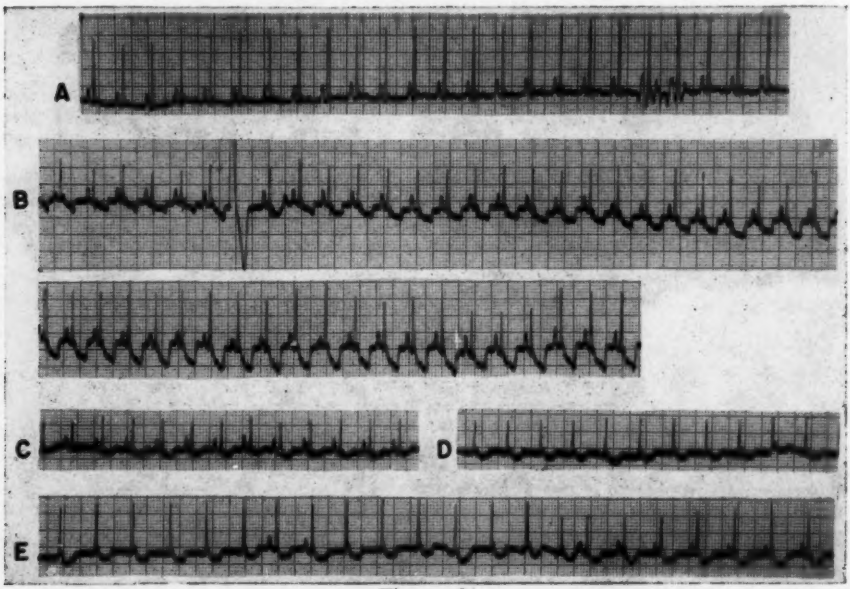

Figure 3

Electrocardiograms from Dog 10 (all lead II). A is a control tracing. B (the next two strips) is a continuous lead recorded at the time coronary artery was accidentally cut, 15 minutes after ligation of the sinus node artery, and shows progressively increasing ventricular ischemia; C is at 20 minutes, D at 22 minutes and E at 25 minutes. See Table I for description.

in the myocardium elsewhere than in the left ventricle need not be associated with any fall in intraluminal pressure of the coronary arteries, and consequently collateral flow may occur at an optimal rate. Dog #10 is an example, having developed sinus arrest only after the occurrence of ventricular ischemia.

In cardiac surgical procedures which compromise flow not only in the sinus node artery but also of any potential collaterals in the area, it can be anticipated that more prolonged or permanent disturbances in atrial pacemaking may occur. This is particularly true of those procedures which involve "cleavage" of the upper interatrial septum, a maneuver which not only damages the sinus node artery but also divides a region across which many of the important anastomoses must pass.[8]

SUMMARY

The sinus node artery was ligated in 16 dogs. Most of the electrocardiographic changes observed in 9 of the 16 dogs were non-specific and transient.

REFERENCES

1. Halpern, M. H.: Arterial supply to the nodal tissue in the dog heart, Circulation 9:547, 1954.

2. Botti, G., Visioli, O., Uleri, G. and Contorni, L.: Effetti immediati della legatura della "arteria del nodo del sono" su la funzione ritmica sinusale nel cane. Rass. Fisiop. Cl. Ter. 29:149,178, 1957.

3. Pianetto, M. B.: The coronary arteries of the dog. Am. Heart J. 18:403, 1939.

4. Kazzaz, D., and Shanklin, W. M.: Coronary vessels of dog demonstrated by colored plastic (vinyl acetate) injections and corrosion, Anat. Rec. 107:43, 1950.

5. Meek, W. J., Keenan, M. and Theisen, H. J.: Auricular blood supply in the dog. I. General auricular supply with special reference to the sino-auricular node, Am. Heart J. 4:591, 1929.

6. Moore, R. A.: The coronary arteries of the dog, Am. Heart J. 5:743, 1930.

7. James, T. N., and Burch, G. E.: The atrial coronary arteries in man, Circulation 17:90, 1958.

8. James, T. N., and Burch, G. E.: Topography of the human coronary arteries in relation to cardiac surgery, J. Thoracic Surg. 36:656, 1958.

TEMPORARY AORTIC BY-PASS BY MEANS OF A MECHANICAL DEVICE*

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In operations of the aorta, in which temporary occlusion of the thoracic segment is done, the presence or absence of adequate collateral circulation must be considered in order to avoid ischemia of the lower parts of the body and the further complications of renal[15] and medullary damage.

The aortic coartation of the so-called adult type is almost the sole exception in which collateral circulation is usually maintained adequately, and the aortic clamping of the thoracic segment of the arorta is possible for a long period of time without danger of lower ischemia.

In other aortic operations the surgeon has three ways of avoiding the ischemia of the lower parts of the body: a) Short duration of aortic clamping, b) Use of Hypothermia, and c) Use of temporary by-pass systems.

a) Short duration of aortic clamping.[3] The aortic clamping alone is used when it is possible to predict that it will not be necessary to clamp the aorta for a long period of time. This is, of course, the easiest and quickest way. Nevertheless, there can always appear unexpected complications which prolong the time of aortic occlusion.

b) Use of Hypothermia.[3,6] This method prolongs the safe period of aortic occlusion, but the occurrence of ventricular fibrillation and other complications of hypothermia is a relative inconvenience.

c) Use of temporary by-pass systems. — By this means it is possible to prolong for a very long time the safe period of aortic occlusion. With this in mind homografts, heterografts, and plastic by-pass systems have often been used.[1,4-14] A disadvantage, however, of suturing grafts that may be used in the by-pass systems is that some injury of the aorta itself may result which makes the operated area more difficult to repair. Another disadvantage is that the suturing procedure prolongs the operating time.

The purpose of this work is to describe the use in animals of a temporary by-pass by means of a mechanical device, with the view in mind of later human employment.

METHODS

This mechanical device consists of 3 pieces, (Figure 1) (a) Cannula with a fixed curved plate attached to the lower part. (b) Superior mobile curved plate, and (c) Nut.

The cannula is attached to a Tygon tube previous to the procedure, then the attached lower plate is introduced through a small aortic incision of 1 cm and the

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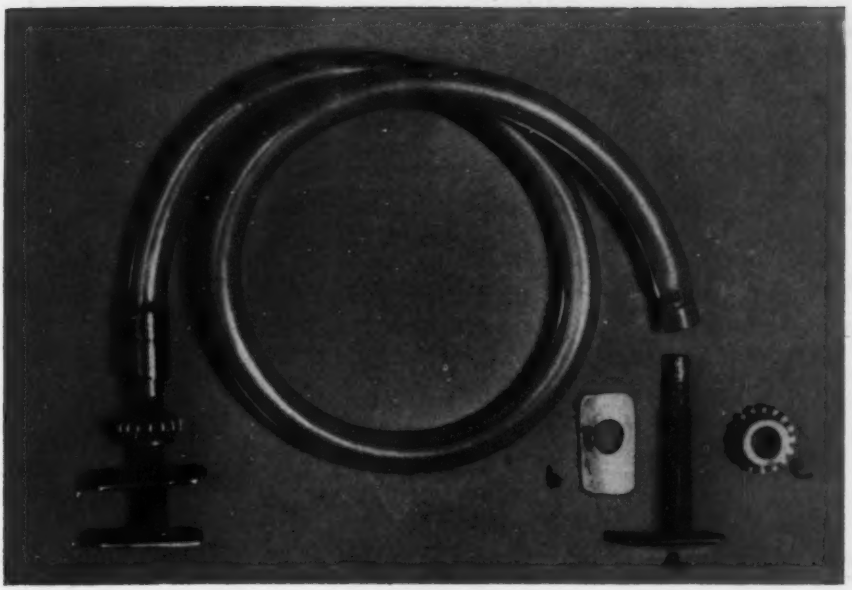

Figure 1

Mechanical device for temporary aortic by-pass. (a) Cannula with a fixed curved plate attached to the lower part. (b) Superior mobile curved plate. (c) Nut.

superior mobile plate is placed down, holding the aortic wall between the two plates, by tightening the nut.

Eleven healthy adult dogs were used. They were anesthetized by intravenous administration of sodium pentobarbital, and tracheal intubation was carried out. After heparinization, the femoral artery was cannulated and connected to a manometer for blood pressure recording.

Two incisions in the left thorax were made, one through the fourth, and the other through the seventh interspace. The aorta was dissected free from the origin of the subclavian artery to the first pair of aortic intercostal arteries, and two aortic clamps were applied.

The fixed curved plate of the lower part of the cannula was inserted into the aorta through a 1 cm. incision in the clamped aorta. The Tygon tube was clamped and the aortic clamps removed.

The same procedure was done in the lower part of the thoracic aorta after ligating one pair of intercostal arteries.

The time employed in the insertion of the cannulas varied between one and eight minutes, the average being five minutes. Some details not taken into account made insertion time longer in the first experiments. In the last three dogs, this time was shortened to an average of 2.5 minutes.

In all the experiments, mean femoral blood pressure was observed: a) Before applying the by-pass, b) Afer cross-clamping the aorta, c) With by-pass in place, d) With by-pass and aortic cross-clamping. Results are shown in Table I.

Table I

Mean Femoral Blood Pressure During the Experiments.

NUMBER OF DOGS	BEFORE BY-PASS	AORTA CLAMPED	WITH BY-PASS AORTA OPENED	BY-PASS ALONE AORTA CLAMPED IN THE MIDDLE
1	100	20	80	55
2	120	20	100	70
3	110	14	100	78
4	100	16	60	50
5	105	15	90	*80
6	95	18	90	*85
7	90	20	80	68
8	112	15	95	80
9	110	15	95	90
10	80	10	70	65
11	80	10	70	50

As can be noted in all the above mentioned experiences, the mean femoral blood pressure was maintained at an average of 70 mm. Hg., the lowest being 50 mm. Hg. which is within safety limits. In only two dogs blood was replaced (5 and 6); as can be seen in these experiments the blood pressure with the by-pass alone is very close to the pre-experimental level.

SUMMARY

In cases of operations of long duration on the thoracic aorta, it is often useful to employ a temporary by-pass system in order to avoid ischemia of the lower parts of the body. For this procedure homografts, heterografts, and plastic grafts has often been used.

The purpose of this work is to demonstrate in animals the use of a temporary aortic by-pass by means of a mechanical device with the view to latter human employment.

Experiences with eleven dogs are discussed in terms of ease of application and mechanical efficiency.

REFERENCES

1. Byron, F. X., Fields, J., Hood, R., and Foster, A.: Use of rigid prosthesis in vascular surgery, J. Thoracic Surg. 34:423, 1957.

2. Chamberlain, J. M., Klopstock, R., Parnassa, P., Grant, A. R., and Cincotti, J. J.: Use of shunts in surgery of thoracic aorta, J. Thoracic Surg. 31:251, 1956.

3. Cooley, D. A., and DeBakey, M. E.: Resection of the thoracic aorta with replacement by homograft for aneurysms and constrictive lesions, J. Thoracic Surg. 29:66:216, 1955.

4. Cross, F. S., Hirose, Y., Jones, R. D., Hudack, S. S., and Kay, E. B.: Evaluation of mechanical shunt to bypass segments of the thoracic aorta including the arch, S. Forum 6:166, 1955 (c1956).

5. Cross, F. S., Kay, E. B., and Jones, R. D.: Simple shunting technique for surgery of aortic and pulmonary valves and proximal great vessels; experimental study, J. Thoracic Surg. 28:229, 1954.

6. DeBakey, M. E., Creech, O., Jr., and Morris, G. C., Jr.: Aneurysm of thoraco-abdominal aorta involving the celiac, superior, mesenteric and renal arteries. Report of 4 cases treated by resection and homograft replacement, Ann. Surg. 144:549, 1956.

7. DeBakey, M. E.: Changing concepts in thoracic vascular surgery, J. Thoracic Surg. 38:145, 1959.

8. Gerbode, F., Braimbridge, M., Osborn, J. J., Hood, M., and French, S.: Traumatic thoracic aneurysms: Treatment by resection and grafting with the use of an extracorporeal bypass, Surgery 42:975, 1957.

9. Johnson, J., Kirby, C. K., and Lehr, H. B.: Method of maintaining adequate blood flow through the thoracic aorta while inserting an aorta graft to replace an aortic aneurysm, Surgery 37:54, 1955.

10. King, H., and Shumacker, H. B., Jr.: Mechanical by-pass during thoracic aortic cross-clamping, J. Thoracic Surg. 34:485, 1957.

11. Lam, C. R., and Aram, H. H.: Resection of the descending thoracic aorta for aneurysm; report of use of homograft in case and experimental study, Ann. Surg. 134:743, 1951.

12. Mahorner, H., and Spencer, R.: Shunt grafts; method of replacing segments of aorta and large vessels without interrupting circulation, Ann. Surg. 139:439, 1954.

13. Schafer, P. W., and Hardin, C. A.: Use of temporary polythene shunts to permit occlusion, resection and frozen homologous graft replacement of vital vessel segments; laboratory and clinical study, Surgery 31:186, 1952.

14. Storey, C. F., Nardi, G. L., and Sewell, H. H.: Traumatic aneurysms of the thoracic aorta; report of 2 cases, one successfully treated by resection and graft replacement with the aid of a shunt, Ann. Surg. 144:69, 1956.

15. Szilagyi, D. E., Smith, R. F., and Whitcomb, J. G.: The kidneys in surgery of the abdominal aorta, A.M.A. Arch. Surg. 77:538, 1958.

REFRACTORY HEART FAILURE

ELLET H. DRAKE, M.D.*

Patients with congestive heart failure will show considerable variation in their individual response to the conventional therapeutic methods. Moreover, a specific patient will vary in individual responsiveness from time to time, and, under certain conditions, may cease to respond or actually to regress while still under intensive

ETIOLOGIC FACTORS IN RESISTANT HEART FAILURE

Cardiac

1. Advanced cardiac disease
2. Active rheumatic carditis
3. Arrhythmia
4. Silent or masked myocardial infarction
5. Myocardial aneurysm
6. Bacterial endocarditis
7. Constrictive pericarditis
8. Unsuspected valvular disease
   (a) Tricuspid stenosis and insufficiency
9. Obscure cardiac disease
   (a) Myocarditis
   (b) Amyloidosis
   (c) Lupus erythematosis
   (d) Polyangitis
   (e) Fibroelastosis
   (f) Hemochromatosis
   (g) Tumors
   (h) Carcinoid syndrome
   (i) Sickle cell disease
   (j) Scleroderma
   (k) Dermatomyositis
   (l) Muscular dystrophy
   (m) Polycythemia vera
   (n) Sarcoid
   (o) "Idiopathic hyper."
   (p) Marfans
   (q) Nephritis

Pulmonary

1. Multiple pulmonary emboli
2. Chronic pulmonary disease (cor pulmonale)
3. Pulmonary thrombosis
   (a) Mitral stenosis
   (b) Auricular septal defect
4. Pulmonary A-V fistula
5. Localized interlobar pleural effusion and regular effusions

Therapeutic

1. Inadequate digitalis
2. Digitalis intoxication
3. Excessive sodium intake
   (a) Diet
   (b) Drinking water
   (c) Private wells
4. Excessive diuretic therapy
   (a) Hypochloremic alkalosis
   (b) Hyperchloremic acidosis
5. Excessive loss of salt
   (a) Vomiting
   (b) Diarrhea
   (c) Low salt diet
   (d) Paracentesis
6. Excessive physical activity
7. Excessive mental stress and emotional activity
8. Blood dilution syndrome (water intoxication)

Figure 1

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Hypermetabolic

1. Hyperthyroidism
2. Anemia
3. Infection and febrile states
4. Liver dysfunction
   (a) Laennec's cirrhosis
   (b) Cardiac cirrhosis
5. Malnutrition
   (a) Beriberi
   (b) Hypoproteinemia
6. Arteriovenous communications
   (a) Systemic A-V fistula
   (b) Paget's disease
   (c) Chronic pulmonary disease

Endocrine

1. Hyperthyroidism
2. Hypothyroidism
3. Hypercorticalism
   (a) Exogenous
   (b) Cushing's syndrome
   (c) Aldosteronism ?
4. Pheochromocytoma
5. Posterior pituitary - A.D.H. and Aldosterone

Lowered Renal Blood Flow

1. Severe intrinsic renal disease
2. Lowered cardiac output
   output down 40%, R.B.F. down 70%
3. Renal vasoconstriction from anuria
4. Local mechanical
   pressure from large collection of ascites

Figure 1 (Continued)

therapy. In this latter state, the patient is often referred to as refractory or intractable. We perfer the former term because of its more optimistic connotation. I wish to discuss first some of the etiologic factors which are important in bringing about this state of relative refractiveness or resistance and then to take up some of the specific methods of management.

For the sake of convenience, I wish to group the important etiologic factors in six categories (Figure 1). It should be emphasized that the various entities mentioned may, in themselves, be a specific underlying etiology for the heart disease or may simply be a complicating or aggravating factor which is acting in conjunction with the basic etiology.

First among the cardiac factors, we have listed Progression of the Underlying Cardiac State.[1] Since most heart disease leading to a state of congestive failure is progressive, it is logical to assume that this progression itself may eventually lead

to a state of refractiveness. But we should not be too quick to adopt this convenient explanation for a patient whose condition is deteriorating until a careful search has been made for other factors which may possibly be reversible. A period of rheumatic activity is frequently responsible for refractiveness to treatment in a patient with rheumatic heart disease. In addition to the common laboratory aids to diagnosis and the EKG, the onset of auricular fibrillation, and the appearance of low-grade fever may be a tipoff that rheumatic activity is present. Arrhythmias and atypical myocardial infarcts will often cause exacerbations of failure and should constantly be kept in mind. Figure 2 shows the x-ray of a man who had gone into

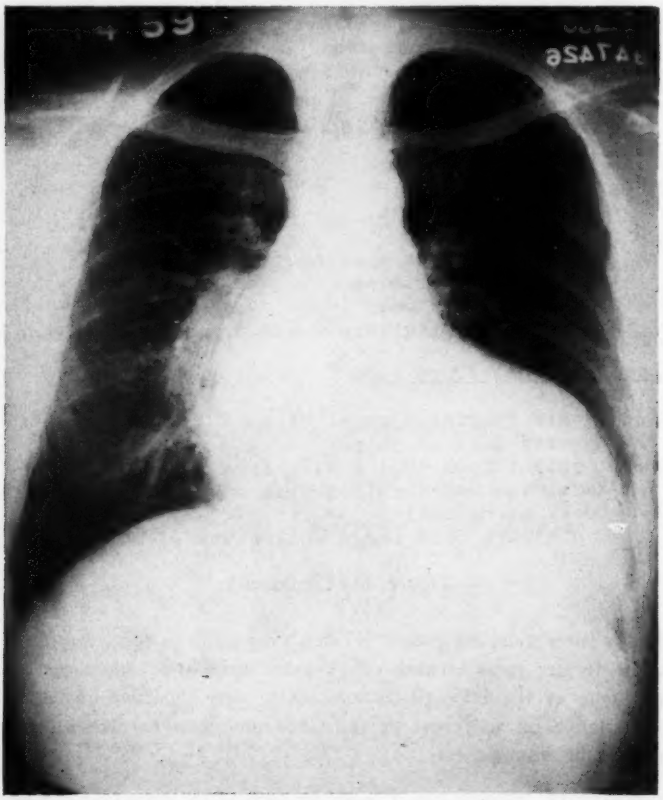

Figure 2

Distortion of the left lateral heart border is due to the presence of a large ventricular aneurysm.

marked congestive failure several months after a rather severe myocardial infarct and whose condition was one of progressive deterioration despite the use of all available medical measures. A myocardial aneurysm was suspected clinically, and despite the fact the fluoroscopic examination was not entirely confirmatory, the patient was subjected to radical surgery for removal of the useless muscle despite

his critical state. At operation, a large part of the ventricular wall which included the aneurysm was resected with a resulting improvement in his state of compensation. Bacterial endocarditis should always be kept in mind in patients with valvular lesions whose failure is not responding to treatment, especially in those patients in the older age groups who have lesions of the aortic valve. Fever will often not be a prominent part of the picture, and the offending organism must be diligently sought for especially in those cases where prophylactic antibiotics are being given to prevent pulmonary infection as a result of stasis. In those patients in whom a right sided lesion is a possibility, cultures of pulmonary artery blood, taken by a catheter, should be considered. Constrictive pericarditis is sometimes difficult to diagnose, but the gratifying response which follows a successful surgical treament in a patient not responding to digitalis and other medical measures makes it well worth the effort to keep this condition constantly in mind. It is not necessary that the patient have a small cardiac shadow on x-ray or that the pulsations necessarily be abolished over the entire heart border for this lesion to be present. Patients with previous cardiac hypertrophy and those with associated pericardial effusions may show considerable enlargement to x-ray and physical examination, and the offending constricting band may involve only a small segment of the heart surface. Among unsuspected valvular lesions, those of the tricuspid valve may be especially troublesome. They may account for the lack of an effective response in a patient who has undergone a successful mitral commissurotomy. For the sake of completeness, we have listed several obscure cardiac diseases which may lead to conditions of resistant failure. Some of these involve the heart primarily, such as fibroelastosis and primary amyloidosis. Others may affect the heart indirectly such as sickle cell disease while still

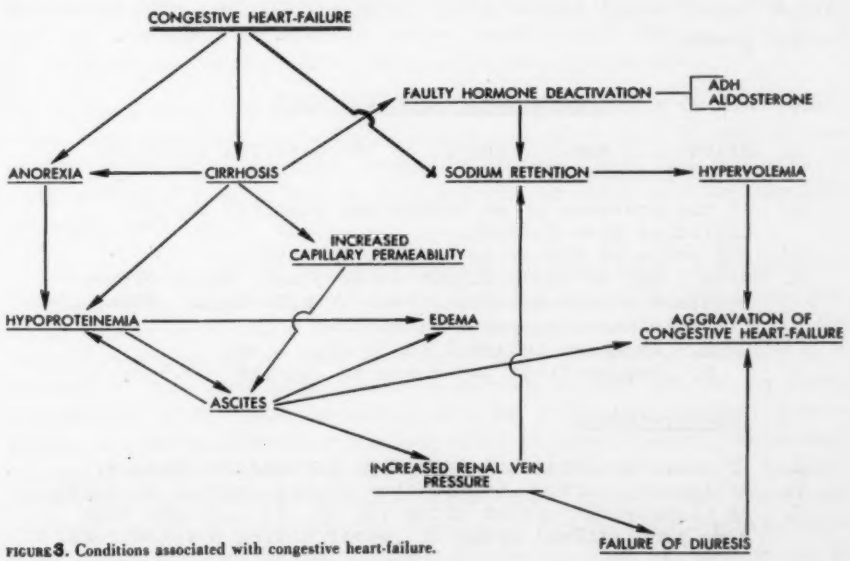

FIGURE 3. Conditions associated with congestive heart-failure.

Figure 3

others may do both such as Marfans which may not only produce valvular lesions but also contribute toward the intractability of failure by destroying the normal elasticity of the aorta.

Many hypermetabolic states are important aggravating factors leading toward resistant congestive heart failure. Latent hyperthyroidism should be suspected in any patient in whom fibrillation does not respond satisfactorily to Digitalis, who shows bouts of transient glycosuria, or in whom the complexion is pink in contrast to the usual sallow washed-out complexion of the cardiac in failure. Protein bound iodine determinations and uptake studies will help to confirm or deny such a clinical opinion. Anemia, regardless of the cause, will aggravate congestive failure if the hemoglobin value falls to a range of 60% or less. Infections and febrile states are extremely important both in precipitating bouts of congestive failure and in contributing to a state of refractiveness. In a recent series of 300 cases of congestive failure admitted to a British hospital, respiratory disease was present in 167 cases and was the precise cause of failure in 156 cases.[2] It was especially important in older people. The role of the liver in congestive failure is a complex one. Figure 3 will illustrate some of the possible pathways by which it plays a part in contributing to a refractory state. Arterio-venous communications with their associated many-fold increase in circulating blood volume add an additional load to the heart which cannot be successfully combated by specific treatment directed toward cardiac function alone. Multiple pulmonary emboli are responsible for a resistant or refractory state in many cardiacs undergoing therapy for congestive failure and thrombosis of a major vessel is not an unusual terminal event in patients suffering from mitral stenosis or intracardiac shunts. Large localized fluid collections will seriously impair cardiac function in the failing patient and should be removed wherever possible.

ACETYL STROPHANTHIDIN TEST

1. Dilute 1.2 mgm. (2 amps.) of the material to 20 cc. in 5% glucose and H_2O.
2. In the presence of an arrhythmia possibly due to digitalis give K first.
3. Run strip of EKG prior to each injection.
4. Give 5 cc. of the dilution every 5 min. until either evidence of therapeutic effect or mild toxic effects. (If intoxication suspected either
   a. Increase interval to 10 min. - or
   b. Reduce first two doses to .15 mgm. (2.5 cc.).

Interpretation

1. If toxicity after 1st injection presume overdosage.
2. If toxicity after .6 mgm. then digitalization adequate.
3. If therapeutic effect after .6, .9, or 1.2 mgm. then give proportional doses of longer acting preparations.

Figure 4

It is somewhat ironic that many therapeutic measures may, in themselves, contribute to resistance in the patient with cardiac failure. Often it is very difficult to tell clinically whether a patient with nausea, vomiting and an arrhythmia may be suffering these manifestations on the basis of the congestive failure alone or whether he may be over digitalized. Figure 4 shows the details of the acetyl strophanthidin test which, while not without its dangers, may sometimes be justifiably employed to determine the status of digitalization.[3] If over digitalization is suspected, potassium should be administered prior to the test and the dosage should be reduced as indicated. Excessive intake of sodium is not often overlooked in treating the patient with congestive failure, but I would like to call attention to the possible hazards existing to the patient who obtains his drinking water from a private well. Many of these are quite high in sodium content. Water softeners may also present a similar hazard and either of these two potential sources of sodium may explain the reason why a patient will do well while hospitalized and then rapidly slip back to failure when he returns to his home.

Certain metabolic factors should be mentioned briefly. Hyperthyroidism has been discussed previously and is included here only for completeness. It is doubtful whether hypothyroidism may, in itself, be actually the cause for their congestive failure, though some patients made artificially myxedematous in the treatment of angina pectoris develop large pericardial effusions which may actually seriously impair myocardial efficiency. Excessive adrenocortical activity may either be of an exogenous nature or occur with a Cushing's syndrome. There is conceded to be some adrenal autonomy in the production of aldosterone although this substance may, of course, also result from increased posterior pituitary activity acting through the adrenal cortex. Aldosterone secretion is influenced by the volume of body fluids and any reduction of extracellular volume occurring with bleeding, drainage of ascitic fluids, salt depletion and so forth, will increase its production.

Lowered renal blood flow will be found in cases complicated by severe intrinsic renal disease which may not be amenable to therapy, but it may also occur in states of lowered cardiac output, renal vasoconstriction from anuria, and as a result of local mechanical pressure from a large collection of acities or other conditions potentially reversible.

In discussing some of the specific methods of management of a case of refractory failure, the question of disordered electrolyte metabolism, which may aften be found in these states, must be considered.[4,5,6] Figure 5 shows the usual "electrolyte patterns" which are said to result from congestive failure alone or secondary to its treatment. I bring these to your attention primarily to point out that the clinical manifestations of the various syndromes are quite similar and I seriously question whether they can be diagnosed on that basis alone. Moreover, chemical determinations will not be conclusive except in a few instances, since they do not supply necessary information to allow complete separation of dilution and depletion states. The dilution syndrome will often be found to develop spontaneously in patients with congestive failure while sodium depletion and hypochloremic alkalosis are often the result of the intensive administration of diuretics, particularly mercurials. In practice, these specific

ELECTROLYTE PATTERNS IN
RESISTANT CONGESTIVE FAILURE

Name	Chemical	Cause	Clinical	Therapy
Sodium Depletion "Low Salt Syndrome"	Na Cl Bicarb. ↓ K Azotemia Acidosis	Low Na Intake Diuresis Diarrhea Sweating Thoracentesis Paracentesis	Anorexia Nausea and vom. Apathy drowsiness Restlessness Confusion, coma Muscular weakness Cramps	Salt by mouth Conc. saline IV 200 cc 5% b.d. Rx - acidosis Bicarb. lactate K.
"Dilution Syndrome" (May develop spontaneously in CHF with conservative management).	Na ↓ Cl ↓ (Total body stores normal or increased) azotemia	Low Na Intake Unrestricted fluids Reduced renal function Anti-diuretic hormone	Insidious onset Apathy Anorexia Nausea and vom. Drowsiness Psychosis Confusion, coma	Restrict fluids K. Steroids
Hypochloremic Alkalosis	Cl ↓ Na ↓ Bicarb.↑ alkalosis	Diuretics anorexia vomiting	Apathy Anorexia Weakness Drowsiness Mental confusion	Ammon. Chloride orally IV 1% in 5% glucose (Not over 100 cc in 1 hour or 1500 cc per day) K. and Ca. Diamox
Respiratory Acidosis	Same with acidosis	Always assoc. with pulmonary disease. CO_2 retention		
Hyperchloremic Acidosis	Cl ↑ K Bicarb.↓ Azotemia	Exchange Resins Diamox NH and Cl Usually renal disease	Hyperpnea Stupor Coma	Mercurial Diuretics Alkaline Sod. Salts

Figure 5

PRACTICAL PROGRAM FOR PATIENT REFRACTORY TO DIURETICS

1. Ease up on the diuretic therapy.
2. Search again for factors which may aggravate failure
   a. Hypoproteinemia
   b. Hydrothorax and ascites.
3. Avoid direct attack on electrolytes when clinical condition of the patient is satisfactory.
4. Re-evaluate the possibility of surgery in eligible cases.
5. Give potassium (even if blood levels are not low). Give 3-4 grams daily. This may produce diuresis and rise in sodium.
6. Try the "Rubin Regime."
7. Hypertonic salt solution.
8. Massive steroid therapy.

Figure 6

syndromes that are so glibly described in the literature, are often difficult to differentiate although we might make one or two generalizations. Most patients with congestive heart failure and hyponatremia do not have a true sodium depletion syndrome and will not benefit from the administration of concentrated salt.[7] Moreover, the unfavorable clinical situation associated with electrolyte disturbance is more often not due to the electrolyte disturbance itself, but rather to a result of the progression of the heart

"RUBIN REGIME"

Effective both for patients with normal electrolyte patterns and those with chloride depletion.

1. Diamox daily for 3 - 4 days.
2. Then - add 10 grams of ammonium chloride daily for 3 - 4 days.
3. Stop diamox but continue ammonium chloride.
4. Give parenteral mercury 48 hours after discontinuing the diamox.

Figure 7

disease and its complications. In Figure 6 we have presented a practical program for approaching these patients with electrolyte disturbances that cannot be readily pinned down as fitting into one or another of the syndrome patterns. Point six refers to the "Rubin Regime" which is detailed in Figure 7 and 8. This program has repeatedly given satisfactory results in our hands, often restoring the response to diuretics and markedly improving the basic failure state. The patient should be watched for excessive acidosis during the time it is administered. Aminophyline may often be used successfully to augment the effect of mercurists. It may be safely administered in 0.5 gram doses diluted to 250 cc. in 5% dextrose, the entire injection made over a 30 minute period, beginning about three hours after the injection of the mercurial.[9]

ANTICOAGULANTS IN RESISTANT CONGESTIVE FAILURE

Thromboemboli found in 30% of patients dying of CHF. In many they are the direct cause.

Indications

1. Rheumatic heart disease with emboli.
2. Thrombophlebitis

Consider also in

1. Massive leg edema with or without areas of inflammation and induration.
2. Increasing dyspnea during the course of congestive heart failure.
3. Appearance of icterus.

Method

Depression of prothrombin activity to 45% satisfactory for prophylaxis. Lower if actual clotting present.

Good anticoagulant service a "must."

Figure 8

Edema in the cardiac patient may often be due in part to a severe state of hypoproteinemia which, for obvious reasons, cannot be successfully combated by dietary intake. Intranveous salt poor albumin may give temporary benefit but this is an expensive measure. We have had considerable success with tube feeding, using small polyetheline tubes through which the food mixture is forced by a "Barron pump." A complete meal is first prepared in a colloid mill and administered over a several hour period, the tube being placed in the duodenum if absorption or vomiting is a problem.[10,11] The feedings on the whole are well tolerated, and the procedure may be adopted for home use employing a blender or even an ordinary kitchen mixer provided the feeding is strained prior to its passage through the pump. The small bore of the polyethelene tube makes it extremely well tolerated for long periods of time.

Thromboemboli are found in 30% of patients dying of congestive heart failure and in many of these, they are the direct cause of the demise. This has resulted in expanding the indications for the use of anticoagulants to include not only those

I-131 IN RESISTANT CONGESTIVE FAILURE

Rationale

1. Reduction in metabolism means reduction in cardiac work.
2. May decrease sensitivity of CV system to adrenergic mediators.
3. May cause alteration in pain perception.
4. May increase rate of development of inter-coronary collaterals.
5. (Raab) Thyroid hormone effects the myocardial metabolism by potentiating hypoxia producing, calorigenic and toxic action of the adreno-sympathogenic cortical amines.

Selection must have

1. Severe disease.
2. Been unsuccessful on medical program.
3. Not in moribund state.

Dosage

1. Blumgart - 15 mc. stat, then 2 subsequent doses at weekly intervals each 5 mc. larger.
2. Jaffe - 6 mc. per week for 5-6 weeks.

Results

Blumgart	Jaffe
24% strikingly improved	53% excellent
30% worthwhile	33% good
46% no benefit	14% not good

Figure 9

patients with thrombophlebitis or rheumatic heart disease with emboli but also other cases with massive latent edema with or without areas of inflammation and induration and even to some patients with increasing dyspnea during the course of congestive failure or those who show the presence of icterus. Depression of the prothrombin activity to 45% is satisfactory for prophylaxis, and this may be depressed further if actual clotting is present. A satisfactory anticoagulant service should be available before such therapy is undertaken. (Figure 8)

Over the past ten years, various clinics have evaluated the usefullness of I-131 in severe angina pectoris and to a lesser extent its use in congestive failure.[12,13,14] Figure 9 outlines the method in which this may be used and the results which have been obtained in two of the larger centers.

Our own results with congestive failure are much less satisfactory than those achieved in angina pectoris. The unpleasant side reactions associated with this therapy considerably limits its usefullness and are a source of concern both to the patient and his relatives. It is mandatory that the patient's status be stabilized before instituting treatment, and it should be borne in mind that the basis underlying disease process is not effected by this treatment. No single dose larger than 20 millicures should be given in order to avoid thyroiditis. We have previously pointed out that I-131 should not be administered to a patient receiving anticoagulants since the isotope acts by producing a hemorrhagic necrosis of the gland.[15]

Recently, the use of the steroids in the management of resistant heart failure has received considerable play in the literature.[16-19] We normally think of the steroids as promoting salt retention and, therefore, contra-indicated in this situation, but actually they may be employed profitably in certain instances to promote water diuresis. Some of the possible theories of their action are listed in Figure 10. The diuresis following the use of steroids is somewhat unpredictable as to its time of occurrence. In some instances, it may not occur until after the drug is stopped, but usually it takes place

USE OF STEROIDS IN THE
MANAGEMENT OF RESISTANT HEART FAILURE

Rationale - (Theories)

1. Increase glomerular filtration rate.
2. Inhibit anti-diuretic hormone.
3. Suppression of secondary aldosteronism.
4. Re-potentiation of diuretic drugs.
5. Anti-inflammatory action as in rheumatic heart disease.

Dosage (not well standardized)
Prednisone - 7.5-25 mgm./day

1. Keep in mind associated disorders e.g. - ulcers, infection, tbc.
2. Continue the basic cardiac therapy.

Figure 10

after 5 to 6 days of administration. In the cases presented by Mickerson and Swale,[18] the serum sodium concentration was uniformly decreased before the institution of steroid therapy and increased and remained at normal level despite a profuse diuresis. It is noted that two of their patients died of pulmonary embolism after one and two weeks of Prednisalone therapy respectively. The usual hazards associated with steroid administration such as ulcers, infection and so forth, are to be kept in mind, and the basic cardiac therapy should be continued during their administration.

We have not had any extensive experience with the use of aldosterone antagonists and what little use we have made of them has been somewhat disappointing. Recent information indicates that the dosage should be revised upwards to 1200 or 1500 mg. a day and this is quite an expensive proposition considering the present market price of the available products. The manufacturers of one preparation (Aldactone)* claim a true synergistic reaction with conventional diuretics and advice the concurrent use of a mercurial or thiazide preparation. Aldactone apparently does not cause potassium secretion and may even offset the potassium loss induced by mercurials or thiazide preparations when used in combination with them. Further work will be necessary to evaluate these products and place them in their proper perspective in regard to the treatment of the patient with refractory congestive heart failure. Finally, a word should be said in regard to the removal of localized deposits of fluid in the edematous cardiac. This is easily accomplished in the case of hydrothorax or ascites but presents a more difficult problem in the case of massive leg edema. We have used multiple punctures, employing a regular hypodermic needle, inserted through a cork to permit the proper depth of penetration. The sites of puncture should first be prepared with a local anesthetic and strict asepsis should be maintained. We have also successfully employed a method employing the introduction of small lengths of polyethelene tubing which are placed through an 18 gauge thin wall needle that is then immediately withdrawn, leaving the tube in place. Several tubes can be placed at strategic points in an edematous extremity, but should probably not be allowed to remain more than twenty-four to forty-eight hours. Here again, asepsis is most important and antibiotics should probably be administered prophylactically to combat the possibility of infection. We have not found the ligation of the inferior vena cava to be a useful procedure in the management of our patients to date, although there are several reports in the literature which advocate this procedure.[20] Wirringer suggests that it is an especially useful procedure to employ preoperatively in certain cases of mitral stenosis possessing an increased risk because of their advanced state of failure.[21] They suggest that this may be followed later under more favorable conditions by the commissurotomy performed as a second stage procedure, in a more fully compensated heart. Lian and his associates state that the most favorable patients for this maneuver are those in whom stasis is limited to the lungs. A less favorable group are those with stasis both in the lungs and in the liver with the least favorable group being those with viscera stasis associated with edema. The operative mortality is high in the series quoted, amounting to 25%, with another author citing a 50% mortality within a six month period. The advocates of this procedure claim prophylaxis against emboli as another advantage and state that it also may be combined with lumbar sympathectomy.

*G. D. Searle and Co.

SUMMARY

Various etiological factors important, not only as the primary causative condition but also as aggravating factors in cases of resistant cardiac failure are reviewed and possible therapeutic approaches to the problem are discussed. Special mention is made of the difficulty in diagnosing with certainty, the various disordered electrolyte patterns. It is felt that special emphysis should be given to the correction of hypoproteinemia when it exists and that steroids and anticoagulants show exceptional promise in many cases. The results with radioactive iodine are, in general, somewhat disappointing and the use of Aldosterone antagonists will require further evaluation before their ultimate usefullness is established.

REFERENCES

1. Keyes, J. W., Drake, E. H., Alvarez, H., Jr., and Breneman, G. M.: Refractory heart-failure, Postgrad. Med. 24:67, 1958.

2. Flint, F. J.: Factor of injection in heart failure, Brit. M. J. 2:1018, 1954.

3. Lown, B., and Levine, S. A.: Current Concepts in Digitalis Therapy, Boston, Little, Brown, 1954.

4. Danowski, T. S., Fergus, E. B., and Mateer, F. M.: Low salt syndromes, Ann. Int. Med. 43:643, 1955.

5. Schwartz, W. B., and Relman, U. S.: Electrolyte disturbances in conjestive heart failure; clinical significance and management, J.A.M.A. 154:1237, 1954.

6. Friedberg, C. K.: Diseases of the Heart, ed. 2, Philadelphia, Saunders, 1956.

7. Uricchio, J. F., and Calenda, D. G.: Failure of hypertonic saline in treatment of hyponatremia and edema in conjestive heart failure, Ann. Int. Med. 39:1288, 1953.

8. Rubin, A. L., Thompson, H. G., Jr., Braveman, M. S., and Luckey, E. H.: Management of refractory edema in heart failure, Ann. Int. Med. 42:358, 1955.

9. Kay, C. F.: Current status of therapy for congestive heart failure, J.A.M.A. 164:659, 1957.

10. Barron, J., Prendergast, J. J., and Jocz, M. W.: Food pump; new approach to tube feeding, J.A.M.A. 161:621, 1956.

11. Barron, J.: Preparation of natural foods for tube feeding, Henry Ford Hosp. M. Bull. 4:18, 1956.

12. Blumgart, H. L., Freedberg, A. S., and Kurland, G. S.: Radioactive iodine treatment of angina pectoris and congestive heart failure, Circulation 16:110, 1957.

13. Jaffe, H. L., Rosenfeld, M. H., Pobirs, F. M., and Stuppy, L. J.: Radioiodine in treatment of advanced heart disease; end results in 100 patients, J.A.M.A. 151:716, 1953.

14. Jaffe, H. L., Rosenfeld, M. H., Pobirs, F. W., and Stuppy, L. J.: Radioiodine treatment of euthyroid cardiac disease; 4 years of experience with 231 patients, J.A.M.A. 159:434, 1955.

15. Freedberg, A. S., Kurland, G. S., and Blumgart, H. L.: Pathologic effects of I131 normal thyroid gland of man, J. Clin. Endocrinol. 12:1315, 1952.

16. Heidorn, G. H., and Schemm, F. R.: Clinical use of corticotropin (ACTH) and adrenal corticosteroids in therapy of intractable edema, Am. J. M. Sc. 229:621, 1955.

17. Riemer, A. D.: Application of the newer corticosteroids to augment diuresis in congestive heart failure, Am. J. Card. 1:488, 1958.

18. Mickerson, J. N., and Swale, J.: Diuretic effect of steroid therapy in obstinate heart failure, Brit. M. J. 1:876, 1959.

19. Newman, D. A.: Reversal of intractable cardiac edema with prednisone (meticorten) J. Florida M. Assoc. 45:778, 1959.

20. Lian, C., Welti, H., and Morgenthaler, F.: Remarques sur la ligature de la veine cave inférieure dans l'insuffisance cardiaque; résultats, indications et contre-indications, Presse méd. 60:313, 1952.

21. Wiringer, P.: Ligature de la veine cave inférieure dans les cardiopathies décompensées; association d'une sympathectomie lombaire droite à l'intervention classique, Acta chir. belg. 51:677, 1952.

ACUTE LEUKEMIA IN ADULTS

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It is the purpose of this communication to review briefly our current experience with acute leukemia in adults at the Henry Ford Hospital. Comparative data on survival and remissions and comments on clinical points of importance make up the body of the report.

MATERIAL

From late 1957 to April 1960, 18 consecutive cases with which the writer has had personal knowledge have been used for the purpose of this study. Of the 18 cases, 11 had the onset of disease after the age of 50, 17 of the 18 occurred after the age of 30, and one, a 16-year-old boy, had a fulminating acute lymphocytic leukemia of two days' duration. No childhood leukemia is included. In addition five cases of acute leukemia, evolving from chronic granulocytic leukemia are discussed separately.

All of the cases reported herein received the standard supportive measures employed today; i.e., blood transfusions, steroids, analgesics, and sedatives. Antibiotics were employed only when evidence of bacterial infection was reasonably clear. In addition 6-mercapto-purine was the specific anti-leukemia medication employed in the great majority of these cases. In some instances death occurred before any effect from this drug was obtained, or before the patient's general condition would permit its use. However, our aim here is not to exclude such unfavorable situations, but to take an honest look at our experience.

The diagnoses according to cell type were as follows: 11 cases of acute granulocytic leukemia, 4 cases of leukemia reticulo-endotheliosis, 2 cases of acute lymphocytic leukemia, and 1 case of the DiGuglielmo syndrome, terminating in acute granulocytic leukemia.

RESULTS

It will be seen from Figures 1 and 2 that at the end of one month 50% of the patients had died with a survival range of 2 days to 17 months. This compares roughly with the median survival of 1.7 months reported by Tivey[1] in 179 cases from the literature, and by Gunz and Hough[9] who found a 1.0 month median survival in 25 cases of their own. Both of these series were computed on a basis of diagnosis to death, as was ours. No other series is strictly comparable because the duration of disease is computed from apparent onset to death. As we can see in Figure 1 the same 179 cases of Tivey computed on this latter basis give a median survival of 3.3 months, and Ellison's[2] cases are, in similar manner, more optimistic. Southam, et. al.[3] reported on 173 cases of acute leukemia of both children and adults, and arrived at a median survival of 3.8 months, based again upon onset to death. Best, Limarzi, and Poncher[4] employed a method of accumulative analysis on a logarithmic time scale, again measuring first symptom to death, and found a median survival of 3.6 months in their 153 cases of acute leukemia in all ages.

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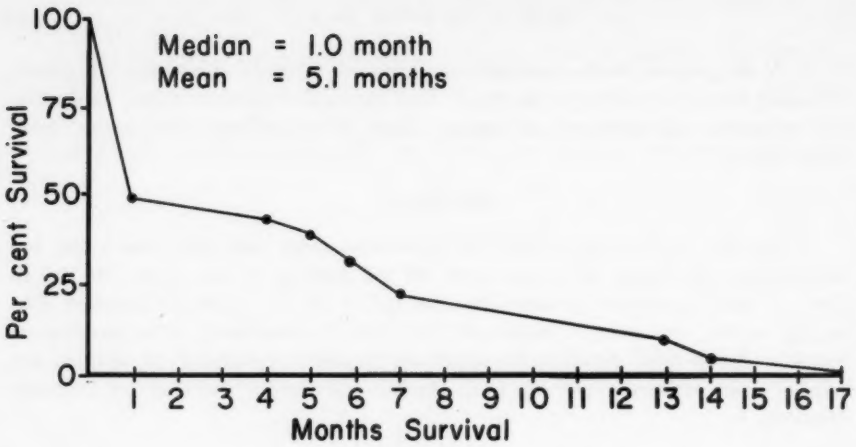

Figure 1

Survival curve in adult acute leukemia, present series. Median survival time is 1.0 month; mean survival time is 5.1 months.

ADULT ACUTE LEUKEMIA

SURVIVAL DATA

Cases	Source	Treated	Median in Months	Based upon:
179	Tivey[1]	No	3.3	Onset to Death
			1.7	Dx. to Death
94	Ellison[2]	Yes	6.5	Onset to Death
54	"	" (NR)*	4.0	" " "
21	"	" (R)**	11.0	" " "
18	H.F.H. ('57-'60)	Yes	1.0	Dx to Death
	"	" (R)	13.0	" " "

*(NR) = No Remission
**(R) = Remission

Figure 2

Those few cases obtaining any sort of remission in adults are diluted by the fulminating, unresponsive cases. In the present series the remission rate of 22% compares favorably with other series (Figure 3). The four cases having remissions are those whose survival exceeded one year.

ADULT ACUTE LEUKEMIA

Cases	Source	Remissions	Per Cent
—	Dameshek[3]	—	10—20
94	Ellison[2]	21	22
18	H.F.H.	4	22

Percentage remissions in adult acute leukemia.

Figure 3

Strangely, 16 of the 18 cases under present analysis occurred in males, and conversely, of the 5 cases of acute leukemia evolving from chronic granulocytic leukemia, 3 were women. The size of the respective groups precludes reaching any conclusion from this finding. Previous large series have arrived at a 2.1 ratio for males over females in adult acute leukemia.

Referral to Figure 4 will help clarify the situation with respect to presenting symptoms. Aside from the nonspecific symptoms of weakness, lethargy, and dyspnea (all manifestations of anemia), which occurred in the majority of patients, it will be seen that almost half presented with some form of abnormal bleeding. In most of these cases this consisted of inordinate bruising and/or petechial formation, but unusual bleeding from recent dental extractions, or other mucous membrane bleeding occurred at the onset of disease also. A history of bleeding manifestations, therefore, is the foremost significant clue when this disease is first encountered; and when the symptoms of anemia and fever occur together with bleeding manifestations, the clinician's index of suspicion for acute leukemia should be high. This point is important only because the average case of acute leukemia has no striking lymphadenopathy, hepatosplenomegaly, or other tumefaction, when first seen.

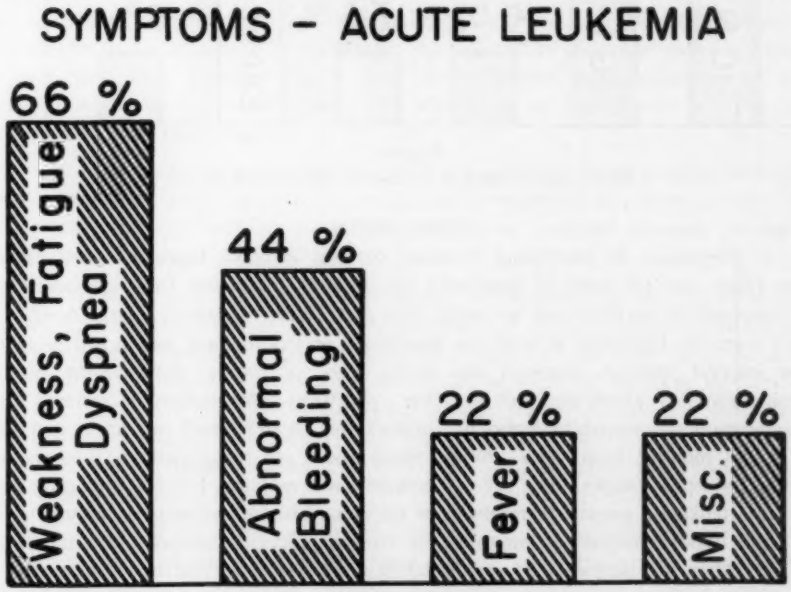

Figure 4

Frequency of symptoms at onset in 18 cases of adult acute leukemia.

Bleeding again plays a prominent role as a major cause of death in acute leukemia (Fig. 5). Sixty-eight per cent of the present series succumbed because of fatal hemorrhage, and 41% had fatal intracranial hemorrhage. Overwhelming sepsis account for 26% of the deaths, and one patient had a fatal myocardial infarct at a time when he was extremely toxic from his primary disease.

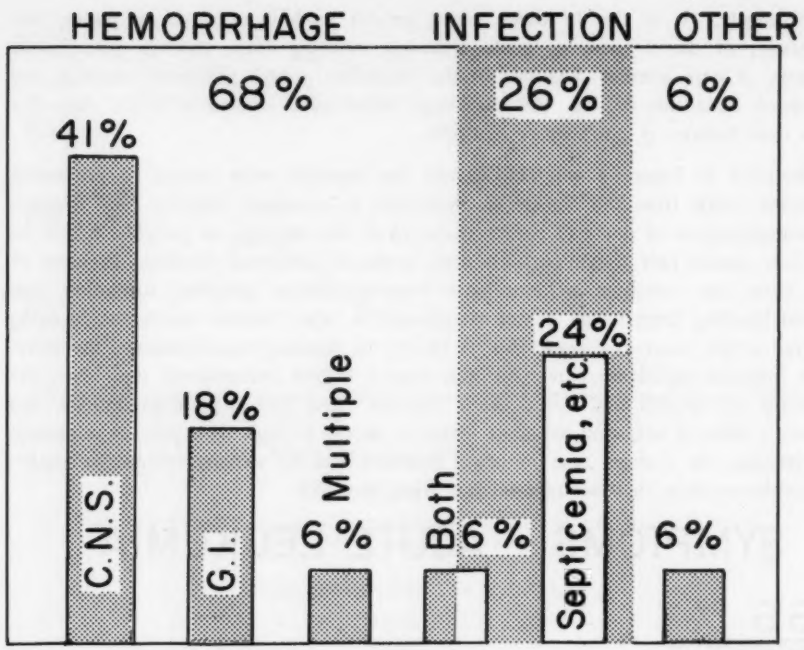

Figure 5
Major cause of death in 17 cases of adult acute leukemia.

DISCUSSION

A. *Prognosis:* In attempting to assess survival in acute leukemia, most authors have fallen into the habit of measuring the duration of disease from apparent onset of symptoms to death. It was not until 1954 that Tivey,[1] reviewing a large number of cases from the literature, stressed the inaccuracy of this method, and urged using the time interval between diagnosis and death. This, of course, shortens the survival considerably (by about one half in Tivey's statistics). Nonetheless, it removes from consideration the uncertain limbo of "preleukemic" states which we know can exist for many months, even years, during which there are recognizable symptoms, but only in retrospect can we surely say that leukemia was evolving. It is true that most cases of acute leukemia present themselves for diagnosis after a period of a month or two of vague non-incapacitating symptoms, but from a practical standpoint it is the period of time after the diagnosis is established that we are interested in, with respect to prognosticating the future and evaluating therapy. Another pitfall to avoid is the use of mean or average survival as a figure to characterize survival in this disease. The survival curve is skewed to the left by the large number of short-lived cases and therefore the median survival figure, which will be considerably shorter than the mean, gives a more accurate picture. McMahon[5,6] stresses this point in recent reviews.

It is apparent from the results of others and those reported herein that the overall prognosis has not changed appreciably with the advent of antimetabolites and other

chemotherapeutic agents. It is only in the individual case where a remission is induced that a definite prolongation of life can be anticipated, and this happens seldom enough. Nonetheless spontaneous remissions (without chemotherapy) in adults are indeed rare, although well documented instances are reported (Moeshlin),[7] and the present incidence of remissions, (about 20%), is a definite sign of progress.

In general there is no prognostic value in determining the leukemic cell type according to Best, et. al.,[4] who attempted to do this in their 153 cases. If any significant difference occurred, it was that their cases of leukemic reticuloendotheliosis had a slightly better median survival than leukemias due to other cell types. This is in contrast to our impression that leukemic reticuloendotheliosis is extremely resistant to myelotoxic agents and results in rapid clinical deterioration. The present series is too small to draw any concrete conclusion on the basis of cell type. The DiGuglielmo syndrome is said to have a better prognosis than other acute leukemias, and our one case of this type of disease bears this out, in that there was a 13-month survival.

McMahon and Forman[5] have attempted to make prognostic significance out of the degree of leukocytosis when the patient is first seen, stating that the subleukemic cases with normal or neutropenic white blood counts tended to do better than those cases presenting with markedly elevated counts. This would seem to be a somewhat artificial device, for it is not uncommon for the total white counts to swing markedly in either direction in a short period of time with no known extraneous influence, and it would therefore be a matter of chance as to what range the counts were in at the time the diagnosis was established.

There is no ready explanation for the male predominance in adult acute leukemia, and there has never been a prognostic significance in the course of the disease favoring one sex over the other,[3] although it has been said that in childhood leukemia the males do not survive as long.

B. *Clinical Aspects:* The present series stresses the frequency of bleeding symptoms at onset, and the frequency of serious intracranial and gastro-intestinal bleeding terminally, the latter sometimes following upon the former by only a few days. At some time during the course of the disease bleeding can be expected to occur in virtually every case. This is a function of depressed platelet formation, and the deleterious effect that fever and general toxicity have upon vascular integrity. It is not unusual to see bleeding manifestations coincide with toxic relapses while the platelet count remains steady, albeit subnormal.

With regard to the occurrence of infection, it can be said that with or without the harmful effect of our drugs on the bone marrow and reticuloendothelial system, these patients are in a precarious situation. Frequently the infection which occurs is so fulminating in character that prompt treatment is to no avail. Nevertheless we feel that there is nothing to be gained by the use of antibiotics prophylactically. There is no drug with a broad enough spectrum of antibacterial action to protect these patients against the odd or resistant organism which will infect the patient. Therefore we still prefer to treat each infection as it occurs. One of our patients, a 64-year-old male, successfully overcame a bilobar Friedländer's pneumonia while simultaneously acquiring a remission in his acute leukemia, and lived comfortably another 11 months.

C. *Acute Leukemia Evolving from Chronic Granulocytic Leukemia:* In our five cases of chronic granulocytic leukemia, terminating in acute leukemia, there was a uniform lack of response to treatment. The occurrence of this unfortunate phenomenon may intervene at any point in the course of chronic granulocytic leukemia. In our cases the chronic form of the disease had been extant from 4 months to 4 years before the evolution to the acute form. Once having become acute, the range of survival was 1 month to 3.25 months with a median survival of 2 months. Serious bleeding was much less common in this setting, with progressive weakness, weight loss, and general deterioration bringing about the demise. Circulatory failure and pneumonia frequently occurred in the last 48 hours of life.

SUMMARY

1. A review of 18 cases of acute leukemia in adults seen at the Henry Ford Hospital between September 1957 and April 1960 is made.

2. Data on survival and a few salient clinical features are presented.

3. A brief review of the pertinent literature is used for comparison.

4. Data on 5 additional cases of acute leukemia evolving from chronic granulocytic leukemia is also included.

REFERENCES

1. Tivey, H.: Natural history of untreated acute leukemia, Ann. New York Acad. Sc. 60:322, 1954.

2. Ellison, R. R.: Management of acute leukemia in adults, M. Clin. North America 40:743, 1956.

3. Southam, C. M., Craver, L. F., Dargeon, H. W., and Burchenal, J. H.: Study of the natural history of acute leukemia with special reference to the duration of the disease and occurrence of remissions, Cancer 4:39, 1951.

4. Best, W. R., Limarzi, L. R., and Poncher, H. G.: Longevity in leucemia, J. Lab. & Clin. Med. 38:789, 1951.

5. McMahon, B., and Foman, D.: Variation in the duration of survival of patients with acute leukemia, Blood 12:683, 1957.

6. McMahon, B., and Clark, D. W.: Incidence of the common forms of human leukemia, Blood 11:871, 1956.

7. Moeschlin, S.: Subacute Paramyeloblasten-Leukämien mit merfachen längeren Remissionen, Deutches Arch. klin. Med. 191:213, 1943-44.

8. Dameshek, W., and Gunz, F. W.: Leukemia, New York, Grune & Stratton, 1958.

9. Gunz, F. W., and Hough, R. F.: Acute leukemia over the age of fifty; study of its incidence and natural history, Blood 11:882, 1956.

CASE REPORT

SYRINGOMYELIA ASSOCIATED WITH ARNOLD-CHIARI MALFORMATION

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Syringomyelia is a tubular cavitation of the spinal cord associated with gliosis in the central portion of the spinal cord about the central canal. Other neurologic conditions or developmental defects have been found associated with this disease. Among these are the Arnold-Chiari malformation, scoliosis, hemivertebrae, fused vertebrae, cervical rib, hydrocephalus, "pigeon breast," platybasia, the Klippel-Feil syndrome, and herniation of cervical intervertebral discs.

The terms syringomyelia and hydromyelia are used interchangeably, although according to Greenfield,[3] cavities which involve more than three segments are considered syringomyelia and those with simple dilatation of the central canal of the cord, hydromyelia. Leyden in 1876 stated that syringomyelia in an adult is a "rest" of a congenital hydromyelia that "cuts itself off" from the central canal. Netsky[5] and also Gardner[2] believe that both conditions co-exist and are clinically indistinguishable.

1891 Chiari[1] found hydromyelia in his three cases (one adult and two infants) of congenital hydrocephalus with cerebellar malformation. This malformation was later (1907) given the name of Arnold-Chiari malformation by Schwalbe and Gredig, after Arnold had described a case with malformation of the hind brain without hydrocephalus but with meningomyelocele in 1894.

A review of the literature revealed that Chiari[1] found hydromyelia in seven infants with Arnold-Chiari malformation and myelomeningocele and hydromyelia in fourteen adults and adolescents with Arnold-Chiari malformation but without myelomeningocele, except in one case. Ingraham and Scott[4] reported twenty cases of Arnold-Chiari malformation in infants. Eight of these cases had hydromyelia. Gardner and Goodall[2] reported fifteen patients with syringomyelia which were found associated with Arnold-Chiari malformation without myelomeningocele. Twelve of these cases had the typical sensory loss. On post-mortem examinations, Greenfield[3] found six cases of syringomyelia associated with Arnold-Chiari malformation. Four of these cases had the characteristic signs and symptoms of syringomyelia. All of these cases were found in adults.

The following case was diagnosed after neurological investigation including myelographic study:

This 21 year old right handed white female reported at the Neurology Clinic of the Henry Ford Hospital with the complaint of weakness and wasting of the muscles of both hands.

The patient stated that the present illness started a year previously. At that time she noticed that she was unable to oppose the thumb and little finger of her left hand.

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This condition gradually became worse so that in a few months she developed difficulty in extending her fingers. A few months later, this was followed by similar difficulty in the right hand. The weakness and wasting of the muscles of both hands gradually, but progressively became worse so that six months prior to our examination she had extreme difficulty holding objects with her left hand.

The past history reveals that she had a "bad fall" when she was eleven years old, and at that time she was found to have a "curvature of the spine". She considered herself a moderate alcoholic drinker. Family history reveals that her father is in a mental institution with a diagnosis of dementia praecox. There is no other nervous disease or mental disorder in the family.

The physical and neurological examination revealed a well-developed, moderately-nourished, ambulatory, alert and cooperative female in no acute distress. The presence of scoliosis was very conspicuous. Blood pressure was 136/80. Her gait was normal. Vestibular and coordination tests were well performed. There were occasional fasciculations observed in the left thenar muscles. There was marked weakness and atrophy of the intrinsic muscles of both hands, more so on the left, producing a "claw-hand" deformity. The left biceps and both deltoid muscles were also slightly weak, without

Figure 1
TOWNE'S VIEW OF SKULL SHOWING ENLARGED FORAMEN MAGNUM

evidence of atrophy. The deep reflexes were symmetrically diminished to absent in the upper extremities. The superfical abdominal reflexes were absent. On plantar stimulation, an equivocal extensor response was obtained on the right, but no response was obtained on the left. No Hoffman sign was present. There were no sensory changes. The cranial nerves were grossly intact.

Routine complete blood count and urinalysis were normal. Blood sugar was 85mg/100ml. Cerebrospinal fluid revealed 16mg/100ml of protein. Skull x-ray showed enlargement of the foramen magnum (Figure 1). Spine x-rays showed scoliosis of the mid-dorsal spine to the right with no intrinsic bone abnormality noted.

The patient was presented before a combined Neurology-Neurosurgery conference, and an early atypical amyotrophic lateral sclerosis and syringomyelia were considered in the differential diagnosis. It was suggested there was a possible basilar impression noted in the lateral skull film (Figure 2). On December 10, 1959 myelography was performed which revealed marked widening of the spinal cord at the cervical region, particularly marked from C.5 through C.7 (Figure 3). Bilateral extramedullary, intradural type defects were also found located posteriorly with their inferior margins at the C.1-2 interspace. These were thought to represent the cerebellar tonsils (Figure

Figure 2
LATERAL FILM OF SKULL SHOWING A SUGGESTION OF BASILAR IMPRESSION

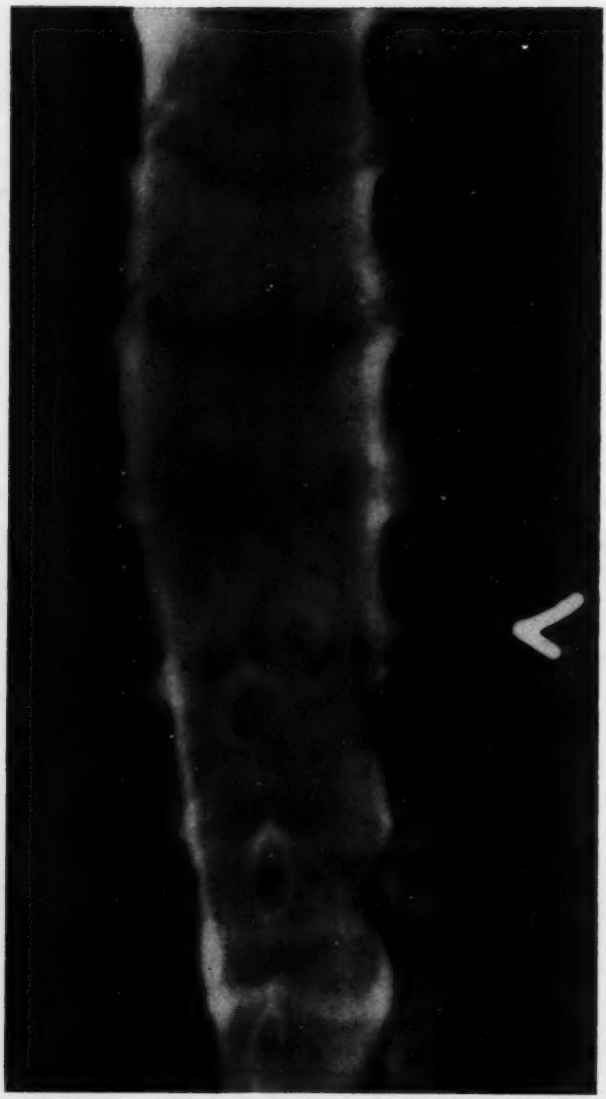

Figure 3
CERVICAL MYEOGRAM OUTLINING SYRINX C5-7

4). On December 12, 1959, suboccipital craniectomy and cervical laminectomy from C.1 through C.3 was performed, with aspiration of a cervical spinal cord syringomyelia and insertion of a small polyethylene tube from the subarachnoid space into the cord cavity. It was thought that there was no evidence of atresia of the outlets of the fourth

ventricle. There was herniation of the cerebellar tonsils which was characteristic of Chiari's Type I malformation.

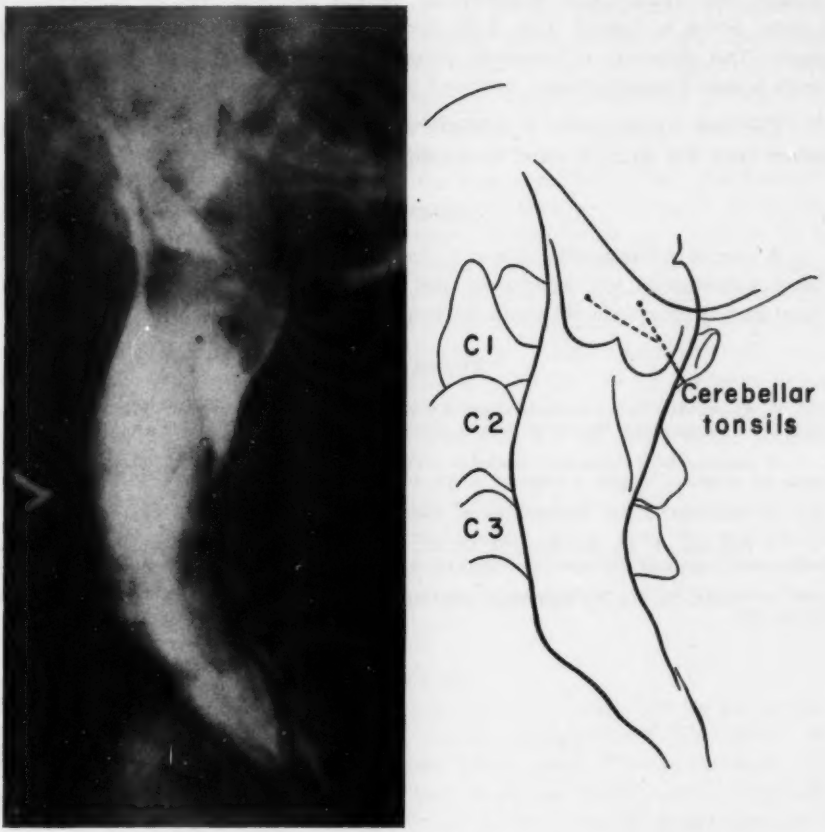

Figure 4

CERVICAL MYELOGRAM REVEALING ARNOLD-CHIARI TYPE I DEFORMITY
NOTE OUTLINE OF CEREBELLAR TONSILS (COMPARE WITH ADJACENT DIAGRAM)

Post-operative course was uneventful and the patient was discharged on December 19, 1959, the neurological condition essentially unchanged.

DISCUSSION

It is noteworthy that this patient presented weakness and atrophy of the intrinsic muscles of both hands without sensory change. This would suggest damage to the posterolateral cell group of the ventral horns of the cervical cord. The lack of dissociated anesthesia probably indicates that the spino-thalamic fibers have to the present escaped damage. We feel that the cavitation arose from the proliferation of glial cells in the region of the central canal of the spinal cord within embryonic rests related

to the imperfect closure of the neural tube. The origin of this particular case of syringomyelia as a malformation is supported by the presence of another developmental anomaly, the Arnold-Chiari malformation. In this particular type of hind brain deformity, which is Chiari's Type I,[1,2,3] there is only the herniation of the cerebellar tonsils. This deformity is frequently called "pressure coning", and this is the type which is most frequently found associated with syringomyelia or hydromyelia in adults.

This case would appear to conform to Netsky's[5] conception, that a "dysplastic" person likely has syrinx if spinal cord symptoms develop.

SUMMARY

A case of syringomyelia without typical sensory changes, associated with Arnold-Chiari malformation, was found in an adult. The presence of dysplasia in a patient with spinal cord symptoms should arouse the suspicion of syringomyelia.

REFERENCES

1. Chiari, H.: Ueber Veränderungen des Kleinhirns, des Pons und der Medulla Oblongata infolge von Congenitaler Hydrocephalie des Grosshirns, Deutsche med. Wchnschr 27:1172, 1891.

2. Gardner, W. J.: Anatomic anomalies common to myelomeningocele of infancy and syringomyelia of adulthood suggest a common origin, Cleveland Clin. Quart. 26:118, 1959.

3. Greenfield, J. G.: Neuropathology, London, Edward Arnold, 1958, pp. 308-316.

4. Ingraham, F. D., and Scott, H. W., Jr.: Spina bifida and cranium bifidum; Arnold-Chiari malformation; study of 20 cases, New England J. Med. 229:108, 1943.

5. Netsky, M. G.: Syringomyelia; clinicopathologic study, A.M.A. Arch. Neurol & Psychiat. 70:741, 1953.

THE HAZARDS OF GASTRO-INTESTINAL INTUBATION

CHARLES D. HAFNER, M.D.* AND JOHN H. WYLIE, JR., M.D.*

A variety of tubes have been employed for various gastro-intestinal problems, particularly in the fields of alimentation, decompression, diagnosis and esophageal tamponade. Originally the chief material used in the construction of these tubes was rubber. However in recent years the development in plastic, especially polyethylene, has made it possible to manufacture tubes which are less irritating to the patient. These various tubes have been the answer to some of the most difficult problems of the grastro-intestinal tract, not only in surgery, but also in medicine. Their judicious use, has been rewarded with the most gratifying results. However we cannot escape the fact that nearly every device which we employ, whether it be an instrument or a medication, is a two-edged sword. The excellent results achieved must be balanced against the occasional complication inherent in the use of such devices. Fortunately the majority of these complications are of minor consideration and are responsible for only minimal discomfort to the patient. However a certain number of these are associated with severe morbidity and even mortality. The purpose of this paper is not to discourage the use of gastro-intestinal intubation, but it is to point out some of the associated hazards, and to encourage the intelligent use of such devices for the management of complicated problems.

Over the past four years we have collected from the surgical services of the Henry Ford Hospital a large variety of complications arising from the insertion of tubes into the gastro-intestinal tract. It is our feeling that these are worthy of note and therefore they are being reported so that others may benefit from this experience. Each has been observed one or more times.

HISTORICAL

In 1790 John Hunter of London reported the use of a stomach tube for the purpose of feeding a patient with paralysis of the muscles of deglutition.[1,2] Throughout the nineteenth century only an occasional sporadic report appeared in the literature concerning the use of gastric tubes. The first four decades of the twentieth century saw the innovation of an entirely new armamentarium for our approach to gastro-intestinal problems. In 1921 Levin[3] described the smooth catheter-tipped tube which is in common usage today and now bears his name. In 1931 Wangensteen[4,5,6] showed the advantage of upper gastro-intestinal decompression in acute mechanical obstruction. Later, in 1933, Wangensteen and Paine demonstrated the advantage of constant suction, over siphonage, in decompressing the upper gastro-intestinal tract. Since that time there has been a tremendous increase in the trend toward the use of various types of tubes for the management of gastro-intestinal problems. This increased trend has resulted from a more thorough knowledge of the underlying patho-physiology of intestinal obstruction; from the performance of a greater volume and greater radicality of major surgery; from a more intelligent approach to the treatment of upper intestinal fistulae; and from increased use of tubular devices for alimentation with liquified foods, as developed and perfected by such investigators as Jones,[7] Snyder,[8] Barron,[9,10] Pareira,[11,12] and others.[13]

*Division of General Surgery.

Since 1934, Miller,[14] Abbott,[14] Cantor,[15] Harris, Honor, Smathers, and other men,[6,19] have developed various types of *long* tubes for small intestinal decompression. In 1941 Silvertsen advised the use of metallic mercury in the balloon of the Miller-Abbott tube to facilitate entry into the duodenum.[6] The Sengstaken-Blakemore tube, for tamponade of bleeding esophageal varices, has been used rather universally in recent years. At many institutions the small-bore, No. 240, polyethylene tube (P. E. Tube), has been used with increasing frequency, primarily for nutritional purposes, but also for small bowel decompression. The future will certainly bring forth different and more complex tubular devices and, of necessity, their associated hazards.

CLASSIFICATION

An anatomical classification of our complications is indicated since various types of tubes can inflict similar trauma as they traverse the gastro-intestinal tract. (Table I).

Table I

COMPLICATIONS OF GASTRO-INTESTINAL INTUBATION.

1. *Nasopharyngeal*
   - A. Pressure necrosis of tip of nose
   - B. Nasal Hemorrhage
   - C. Rhinitis
   - D. Conjunctivitis
   - E. Ear symptoms
   - F. Sinusitis
   - G. Pharyngitis
2. *Laryngotracheal*
   - A. Laryngitis
   - B. Laryngotracheitis—Traumatic
3. *Pulmonary*
   - A. Aspiration pneumonia
   - B. Aspiration asphyxia
   - C. Respiratory obstruction
4. *Esophageal*
   - A. Necrosis
   - B. Perforation
5. *Gastric*
   - A. Perforation (Miller-Abbott)
   - B. Perforation (Gastroscope)
6. *Duodenal—Perforation By Insertion of P. E. Tube At Surgery*
7. *Superior Mesentery Artery—Laceration*
8. *Small Intestine (Jejunum and Ileum)*
   - A. Obstruction
     - 1) Mercury bag
     - 2) Insussussception
     - 3) Secondary to silk suture (P. E. tube)
     - 4) Ulceration and stenosis
   - B. Perforations—Multiple
     - 1) Miller-Abbott tube
     - 2) Silk thread of P. E. feeding tube
9. *Extubation Complications*
   - A. Knotted tubes
   - B. Intestinal Laceration
   - C. Reversed intussussception and accordion effect
10. *Problem of Retained Tube (Unable to remove in usual fashion)*
    - A. Gaseous distention of balloon (Diffusion)
    - B. Liquid distention of balloon (Medication by nurse through incorrect lumen)
    - C. Mercury bag at ileocecal junction
    - D. Mercury bag and silk suture in small intestine (P. E. tube)
11. *Rectal*
    - A. Laceration (pain and hemorrhage)
    - B. Perforation (barium enema tube)
    - C. Perforation (Sigmoidoscope)
12. *Colostomy*
    - A. Perforation with irrigation tube.

NASOPHARYNGEAL

In the nasopharyngeal region a large variety of less serious complications have been experienced with the use of tubes of various types. For the most part these are not serious; however, they are quite distressing to the patient. Pressure necrosis of the tip of the nose should be prevented by avoiding the immobilization of a firm tube tightly against one side of the external nasal orifice. Nasal hemorrhage and irritative rhinitis and pharyngitis can usually be avoided by the gentle insertion of tubes

following adequate lubrication with a local anesthetic ointment. Conjunctivitis, sinusitis, otitis media should be treated symptomatically for the comfort of the patient. Nasal drops, oral drops, anesthetic lozenges and analgesic medication should be used rather freely in the treatment of these unpleasant but less serious complications.

LARNYNGOTRACHEAL

The larynx and trachea of an individual may easily be traumatized during the insertion of intestinal tubes, especially when an uncooperative patient is involved. One dramatic case which we have experienced illustrates this. A 66 year old white female, who was admitted for subtotal colectomy for multiple polyposis, withdrew her Miller-Abbott tube during the night preceding surgery. Attempts to re-insert the tube in this uncooperative patient resulted in such traumatization of the larynx and trachea that the patient barely escaped the necessity of a tracheostomy. This was avoided only through vigorous conservative measures which necessitated the delay of her surgery for two weeks. Although we have experienced numerous instances of laryngitis from prolonged use of tubes, we have not seen the severe stenosing reactions requiring tracheostomy as occurred in 10 cases reported by Iglaner and Molt.[16]

PULMONARY

Aspiration pneumonia and asphyxia is a catastrophe which should and must be avoided. This is one of the most severe hazards with which we are constantly confronted, especially in the field of forced alimentation with liquified foods through intestinal tubes. It is obvious that no forced feeding should be instituted through a tube which is proximal to a level of obstruction. However, it is probably not so apparent to the novice in forced tube feeding that this should never be instituted into the stomach in an unconscious patient, a highly medicated patient, or in anyone who is unable to manage the problem of emesis and regurgitation. Under these conditions the feeding tube must be inserted into the proximal jejunum or beyond a point of obstruction to assure against any significant regurgitation and aspiration.

ESOPHAGEAL

The esophagus is subject to a great deal of traumatization since most tubes employed traverse this segment of the alimentary tract. The complications of the Sengstaken-Blakemore tube[17] should be universally recognized. If prolonged or excessive pressure is maintained in the esophageal portion of the balloon, necrosis and perforation of the esophagus may develop. Aspiration into the tracheobronchial tree of overflow secretions above the esophageal balloon in an acutely ill and debilitated patient is a constant hazard. If the gastric portion of the balloon should deflate for one reason or another, traction externally, as is frequently applied, may cause mechanical obstruction of the larynx as the esophageal balloon is withdrawn.

Figure 1 illustrates an extremely interesting complication which we have encountered in the use of the esophageal tube. This patient developed esophageal varicosities secondary to cardiac cirrhosis which had developed from a chronic constrictive

pericarditis. Following insertion of the Sengstaken-Blakemore tube for hemorrhage the esophagus was pushed forward against a sharp horn of calcific pericarditis. This laceration of the esophagus resulted in fatal mediastinitis in addition to his already existing lethal disease processes.

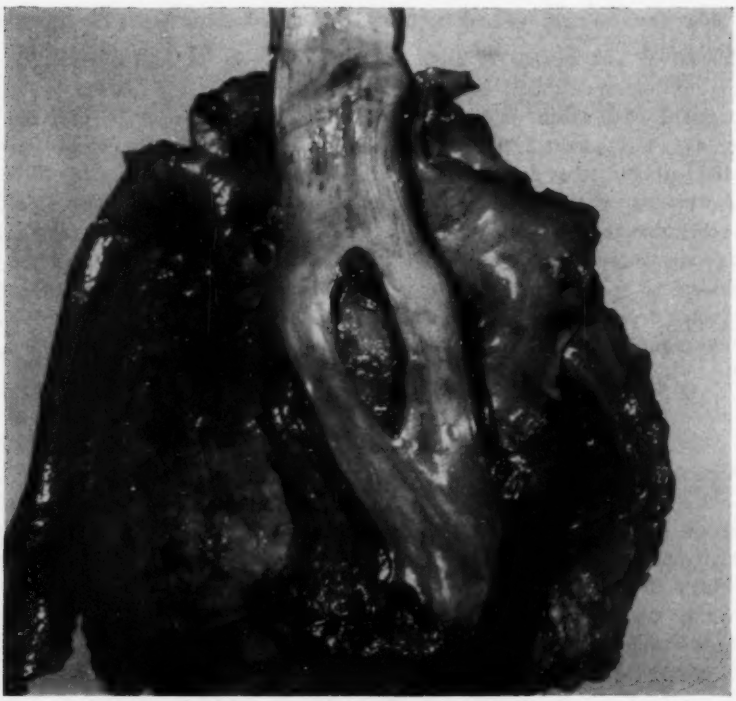

Figure 1
Esophageal laceration against pericardial calcific spur with use of Sengstaken-Blakemore tube.

Perforation of the esophagus is indeed a rare complication from the *ordinary* use of more common gastro-intestinal tubes. However, we have experienced one perforation of the lower esophagus near its junction with the stomach from the prolonged use of a Levin tube.

Diagnostic and therapeutic esophagoscopy has resulted in three perforations in our series and this represents a constant hazard when this procedure is undertaken. Several less serious mucosal lacerations have occurred with the use of the esophagoscope. The cricopharyngeus level presents the most difficult area to intubate, and therefore trauma with perforation is most likely to occur at that point.

GASTRIC

Perforation of the stomach is likewise rare in the ordinary use of gastro-intestinal tubes. However, we have had one patient who experienced a perforation of the stomach

nine months following the insertion of a Miller-Abbott tube. It was impossible to withdraw the Miller-Abbott tube at that time through the nasopharynx. It therefore, was transected with the expectations that this would pass through the intestinal tract and emerge at the rectum. Periodic x-rays, from time to time, encouraged optimism that the Miller-Abbott tube was progressing satisfactorily through the gastro-intestinal tract. Nine months later a portion of the tube was still present in the gastric pouch and was responsible for an acute perforation, requiring surgery for the extraction of the Miller-Abbott tube and closure of the perforation. This indicates that a foreign body of such magnitude which is maintained in the gastro-intestinal tract without good progress in a satisfactory period of time, constitutes an indication for surgical intervention in order to eliminate such severe sequelae as resulted in this case.

Gastroscopy is a more common method of gastric perforation and we have experienced three such cases in our series. Perforation by this method usually occurs high in the stomach near its junction with the esophagus. We believe that gastroscopic examination is by no means an innocuous procedure. It should be performed by an experienced operator under favorable circumstances and with careful manipulation.

DUODENAL

Perforation of the duodenum is indeed a rare complication from the use of Gastro-intestinal tubes. However, in one instance in our series we have experienced a perforation of the fourth portion of the duodenum. This was done at the time of a Billroth I gastrectomy when a polyethylene tube was being inserted beyond the anastomosis into the jejunum for postoperative feeding. A relatively rigid, rubber catheter tube with a fish-mouth slit at the tip is frequently used as an introducer for the insertion of the polyethylene tube into the distal intestinal tract at the time of surgery. Any such method of introduction must be done with extreme care since we believe that this is responsible for a fatal perforation of the fourth portion of the duodenum which was unrecognized at the time of surgery. Other less serious duodenal complications do occur in the form of superficial ulceration and laceration.

SUPERIOR MESENTERIC ARTERY

In one case in our series the superior mesenteric artery was lacerated, resulting in almost fatal hemorrhage. This represents one of our most fascinating and unique complications while using the No. 240 polyethylene feeding tube. The mechanism of trauma here demonstrates one of the most serious and feared complications of such a tube which employs a silk thread for the control of a weighted mercury bag at the end of the tube. This tube must be so constructed that there is no slack between the mercury bag and the end of the tube in order to prevent exposure of the bowel or other tissue to the sawing action of the silk thread. This can be, and is, a lethal weapon analogous to the action of a gigli saw. It should be pointed out here that these polyethylene tubes are so constructed in order to allow release of the mercury bag when peristalic action becomes so great as to cause cramping and obstruction. Following the release of the silk thread and mercury bag, a period of time must elapse before forced feeding is re-instituted. This is necessitated by the fact that the proximal portion of silk thread can be impacted somewhere along the

course of the tube due to the forced feeding, and thereby setting the stage of a potentially lethal circumstance in which a large portion of the taut silk thread is exposed to traumatize the intestinal wall. This has been a real hazard in the writer's experience, for this situation makes it hazardous even for the removal of the tube. In this particular case a bypass duodeno-jejunostomy had been performed in a 26 year old white female for the superior mesenteric artery syndrome. A polyethylene feeding tube inserted at the time of surgery was inadvertently withdrawn and a second tube was reinserted during the postoperative course.

Figure 2

Feeding tube encircling duodenal loop after passing through duodeno-jejunostomy. Note also retained mercury balloon.

Figure No. 2 represents the course that this tube traveled. It traversed the duodenojejunostomy but returned in an anti-peristalic manner through the proximal limb of the jejunum and back through the fourth, third and finally second portion of the duodenum. A locking loop was thus formed around the altered intestinal tract by the polyethylene feeding tube. This resulted in laceration of the duodenum and superior mesenteric artery, and several operations with direct surgery on the artery were required to save the patient from this catastrophe. It should be pointed out at this time that any anatomical situation in which circular continuity of the gastro-intestinal tract has been established is an extremely dangerous one, no matter what type of tube is employed. Therefore, under this condition, precise insertion of

Figure 3

Accordion-like pleating of jejunum on feeding tube causing obstruction of intestine.

gastro-intestinal tubes under careful fluoroscopic visualization is mandatory.

Small intestinal obstruction is a phenomenon which we see with the use of any tube but especially more frequently with the small polyethylene feeding tube.

Figure 3 demonstrates the mechanism by which small intestinal obstruction can occur when peristalsis becomes active on the mercury bag attached to a long intestinal tube which is anchored externally to the patient's nose. Obstructive symptoms resulting from plication of the intestine and finally intussusception are frequently manifested by severe cramping abdominal pain and vomiting. This will occur with the use of any mercury bag intestinal tube which is anchored externally. Therefore we do not allow the Miller-Abbott tube to be anchored securely to the nose as this may result in severe intussusception and its sequelae. It should be pointed out here also that this is the reason for the construction of the polyethylene feeding tube in such a manner, that the mercury bag may be eliminated by the release of the intraluminal silk thread when cramping abdominal pain and other symptoms are first experienced by the patient.

Figure 4 illustrates a chronic intussusception firmly fixed by adhesions resulting in severe cramping pain and vomiting in a postgastrectomy patient.

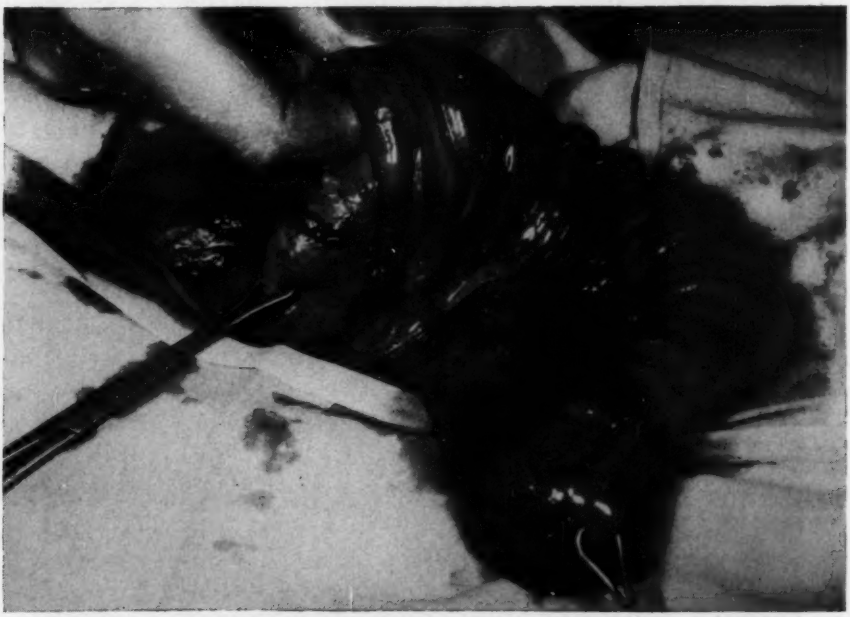

Figure 4

Chronic intussusception produced by feeding tube causing intestinal obstruction.

Several small intestinal obstructions have occurred secondary to retained silk strings after they had been released from polyethylene feeding tubes. It has been

the impression of this observer that the silk strings will pass rather readily through a normal intestinal tract. However, they tend to be retained in the small intestine when intraabdominal inflammatory disease and intestinal adhesions are present. These retained silk strings are frequently associated not only with obstruction but also with multiple perforations of the small bowel. The picture at the time of surgery is one of closely adherent kinked loops of small bowel with minute perforations at the angles of kinking; and, because of the close approximation of the bowel loops these perforations have been rather confined. At least two cases of jejunal ulceration with stenosis secondary to polyethylene feeding tubes have been observed. One case was found at autopsy and the second case required segmental resection of the involved area of jejunum.

We have had one very unique experience in which obstruction with gangrene and multiple perforations of the small intestine was inflicted by injury from a Miller-Abbott tube. The tube in this instance was unable to be withdrawn. Unfortunately, excessive tension was placed upon the tube during the attempts at extubation, and then the tube was anchored securely to the nose. The severe intussusception and accordion effect on the small bowel in this case resulted in gangrene and multiple perforations, requiring resection of a long segment of jejunum and ileum with closure of numerous other perforations in order to save the patient from this unusual injury.

EXTUBATION COMPLICATIONS AND RETAINED TUBES

Extubation complications, per se, that we have experienced have been quite distressing to the patient and are potentially dangerous, but have resulted in very few catastrophes to this writer's knowledge. Some of the more common examples of this condition in our experience have been the withdrawal of knotted tubes with extreme difficulty and discomfort to the patient, mild intestinal lacerations resulting in minimal gastro-intestinal bleeding, and intestinal plication with reversed intussusception which is usually corrected before serious damage has been inflicted. If one is unable to remove the gastro-intestinal tube without undue hazard or damage, one then gets into the problem of the *retained tube*. Some items relating to this category have been mentioned in the preceding paragraphs such as retained silk strings which do not pass, a retained Miller-Abbott tube which caused perforation of the stomach, and the specific instance in which a polyethylene tube encircled the duodenojejunostomy causing laceration to the superior mesenteric artery. However, certain *specific* problems relating to this category have been encountered in our experience. One problem is that of diffusion. Cantor, in 1949, studied the problem of diffusion of gases in and out of rubber ballons, and found this to be a serious one. This is mainly a consideration in tubes with a single lumen since pressure in the double-lumen tubes usually can be released easily. However, one case was reported in the literature in which gaseous distention of a Miller-Abbott balloon resulted from shrinkage of the thread used to secure the balloon.[17] This caused obstruction of both lumens and it was impossible to deflate the balloon. This problem of diffusion has been relatively infrequent, however, in our experience, since precaution is taken to perforate all air-tight mercury bags with a No. 24 gauge needle.

These small holes act as a gas vent as pointed out by Cantor. Another problem which we have experienced is that which is perpetrated by the unsuspecting nurse who injects medication through the incorrrect opening of a biluminal tube. Walker,[18] in 1940, pointed out this difficulty arising from the injection of barium through the channel of the double-lumen tube leading to the balloon. The injection of barium has not been a problem in our experience; however, we have frequently found that various medications have been injected by the nurse through the wrong lumen. This has accounted for some difficult extubations; and in one instance, it was impossible to extubate the tube and mercury bag intact. In this instance, the balloon was exploded by additional pressure and the Miller-Abbott tube was then easily withdrawn. This is a procedure which we do not recommend, since the explosive force of the bag might lead to perforation of the intestine.

Figure 5 illustrates a mercury bag which was retained at the ileo-cecal valve for three weeks, causing symptoms of cramping pain across the abdomen with some rectal bleeding and localized rectal pain especially at the time of bowel movements. In this instance, the silk thread still attached to the mercury bag traversed the entire colon and would emerge from the rectum with bowel movements. On any occasion in which a silk thread presents itself at the rectum we strongly condemn its manual withdrawal since laceration of the colon can easily result from this maneuver.

Figure 5

Retained mercury balloon and string after release from lumen of feeding tube. (Course of string superimposed on x-ray film).

The patient, nurses and resident staff should be thoroughly instructed to merely cut the excess protruding silk thread, and under no circumstance are they to place any tension upon this. In the patient with a retained mercury bag at the ileo-cecal junction, the bag finally ruptured and was passed without severe complications. This is the usual course of events.

RECTAL

Laceration of the rectum with pain and hemorrhage has occurred in our series, especially when tubes and silk sutures have been withdrawn by the patient or uninformed hospital personnel. We have seen one perforation of the rectosigmoid from a tube with an inflatable bulb inserted at the time of a barium enema x-ray. The ordinary straight blunt barium enema tube has caused very little or no difficulties. However, the rubber tube with an inflatable balloon used in children and in patients who are unable to retain enemas is always a potential hazard. This inflation bulb should never be inserted by a technician, but should always be inserted and inflated by a radiologist under direct fluoroscopic vision. We have experienced three perforations of the rectosigmoid region in our series during the use of the sigmoidoscope. This is a relatively infrequent complication; however, as with the use of the esophagoscope and gastroscope, this procedure must be done with care.

COLOSTOMY

One colon perforation occurred in our series when an irrigation catheter was inserted into a colostomy opening. It is indeed a wonder that this accident has not occurred with greater frequency since personnel with variable experience, including the patients, perform this procedure.

SUMMARY

Many of the complications arising through the use of gastro-intestinal intubation which we have experienced in the past four years have been presented. Although many of these appear to be common and trivial, they are, nevertheless, distressing to the patient. Others have been, fortunately, infrequent, but disastrous to the patient. It has been the purpose of this paper, by no means, to discourage the use of tubes in the management of complicated gastro-intestinal problems, but rather it has been the intention to demonstrate some of the hazardous pitfalls which are inherent in the employment of such devices. The indiscriminate use is to be avoided; the ignorant approach is to be condemned; and the intelligent application of gastro-intestinal tubes is to be encouraged.

REFERENCES

1. Paine, J. R.: History of the invention and development of the stomach and duodenal tubes, Ann. Int. Med. 8:752, 1934.

2. Hunter, J.: Case of paralysis of the muscles of deglutition, cured by an artificial mode of conveying medicine and foods into the stomach. In, The Complete Works of John Hunter, S.R.S. Ed. by J. S. Palmer, Philadelphia, Haswell, Barrington & Haswell, 1841, v.4, p. 185.

3. Levin, A. L.: New gastroduodenal catheter, J.A.M.A. 76:1007, 1921.

4. Wangensteen, O. H.: Early diagnosis of acute intestinal obstruction with comments on pathology and treatment, with report of successful decompression of 3 cases of mechanical bowel obstruction by nasal catheter suction siphonage, West. J. Surg., Obst. & Gynec. 40:1, 1932.

5. Wagensteen, O. H.: Therapeutic considerations in the management of acute intestinal obstruction; technic of enterostomy and a further account of decompression by the employment of suction siphonage by nasal catheter, Arch. Surg. 26:933, 1933.

6. Wagensteen, O. H.: Intestinal Obstruction, ed. 3, Springfield, Thomas, 1955.

7. Jones, C. R.: Duodenal feeding, Surg., Gynec. & Obst. 22:236, 1916.

8. Snyder, C. C.: Continuous forced feeding pump, new approach to nutritional therapy, Plast. & Reconstruct. Surg. 8:73, 1951.

9. Barron, J.: Tube feeding with liquified natural foods, Henry Ford Hosp. M. Bull. 1:13, 1953.

10. Fallis, L. S., and Barron, J.: Gastric and jejunal alimentation with fine polyethylene tubes, A.M.A. Arch. Surg. 65:373, 1952.

11. Pareiera, M. D., Conrad, E. J., Hicks, W. P., and Elman, R.: Therapeutic nutrition with tube feeding, J.A.M.A. 156:810, 1954.

12. Pareiera, M. D., and Heeb, M. A.: Transanastomotic feeding following gastric surgery, A.M.A. Arch. Surg. 77:851, 1958.

13. Bisgard, J. D.: Gastrostomy-jejunal intubation, Surg., Gynec. & Obst. 74:239, 1942.

14. Miller, T. G., and Abbott, W. O.: Intestinal intubation; a practical technique, Am. J. M. Sc. 187: 595, 1934.

15. Cantor, M. O.: Intestinal Intubation, Springfield, Thomas, 1949.

16. Iglauer, S., and Molt, W. F.: Severe injury to the larynx resulting from indwelling duodenal tube, Ann. Oto., Rhin. & Laryn. 48:886, 1939.

17. Conn, H. O.: Hazards attending the use of esophageal tamponade, New England J. Med. 259:721, 1958.

18. Walker, I. J.: Discussion of, McKittrick, L. S., and Sarris, S. P.: Acute mechanical obstruction of the small bowel, New England J. Med. 222:611, 1940.

19. Johnston, C. G., Penberthy, G. C., Noer, R. J., and Kenning, J. C.: Decompression of small intestine in treatment of intestinal obstruction, J.A.M.A. 111:1365, 1938.

20. Brenizer, A. G.: Unusual complication of intestinal intubation, New England J. Med. 238:291, 1948.

21. Deterling, R. A., Jr., O'Malley, R. D., and Knox, W.: Intussusception in the adult, with emphasis on retrograde type, A.M.A. Arch. Surg. 67:854, 1953

THE ANALYSIS OF PATHOLOGICAL CRYSTALS BY X-RAY DIFFRACTION

JONATHAN PARSONS*

Crystalline substances are often found in routine biopsy and autopsy specimens. Normally the occurrence of crystalline material in the body is limited to the inorganic salts of bones ond teeth, but under certain conditions crystals may be observed in soft tissue and in various body-cavities. The latter crystals may be exogenous or endogenous in origin and range from the numerous possible metabolic crystals through the unlimited number of foreign materials which may gain entrance to the body. The indentity of these substances may be of no apparent significance or, as in the case of the pneumoconioses, their analysis may have etiological significance. Usually, the identification of the crystals associated with certain diseases will contribute to the accuracy of the diagnosis, aid in establishing pathogenic conditions, and even at times be a guide in the therapy and prevention of recurrence of these disease conditions.

Chemical methods of analysis can be used in some cases but are often of limited value because of (1) the difficulty of extracting the crystals from the tissue, (2) the minute amount of material available for analysis and (3) the complexity of the chemical processes required. The time required for a skilled chemist to perform such an analysis, when possible, is quite excessive. It is the purpose of this paper to show that x-ray diffraction powder analysis, not being subject to the above limitations, often can provide the desired identification of pathological crystalline materials. The description of the x-ray diffraction powder method has been given in an earlier issue of this *Bulletin*[1] and is more completely described by Klug and Alexander.[2]

METHODS AND MATERIALS

When the pathologist observes the presence of foreign matter in a tissue section, crystallinity of the material usually can be established by observing the birefringence of the particles using polarized light. A thin needle-like sliver of tissue, containing the observed crystals, is cut and inserted into a small thin walled glass capillary tube. Fig. 1, illustrates (A) the tube as received, (B) the shortened capillary after insertion through steel pin and with tissue sliver probed to lower end, and (C) the completed mount after removal of funned-end. It should be noted that tissue which has been subjected to histological stains or mounted in a crystalline medium such as paraffin cannot be used. The obvious reason for this fact is that diffracted lines from the latter extraneous crystalline substances will be added to lines from the unknown crystals in the tissue, thus causing confusion in applying normal methods of pattern interpretation. Usually additional portions of tissue, from which a slide has been prepared, are available. Either fresh or formalin fixed tissue can be used, the latter requiring washing in running tap water for about 15 minutes, in order to remove any of the soluble salts due to the formalin.

The equipment used was a Philips x-ray diffraction source unit (Fig. 2). Each sliver of tissue, mounted as described above was fitted into the central chuck (a)

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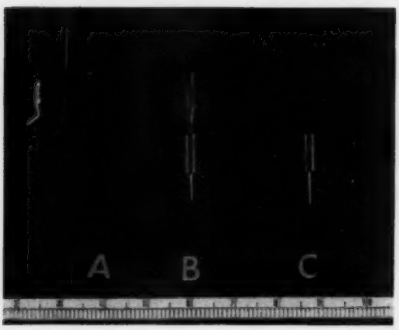

Figure 1

A—glass capillary tube; B—glass capillary tube in pin support, with specimen inserted; C—mounted tube with funnel end removed.

Figure 2

X-ray diffraction source unit with cameras in exposure position.

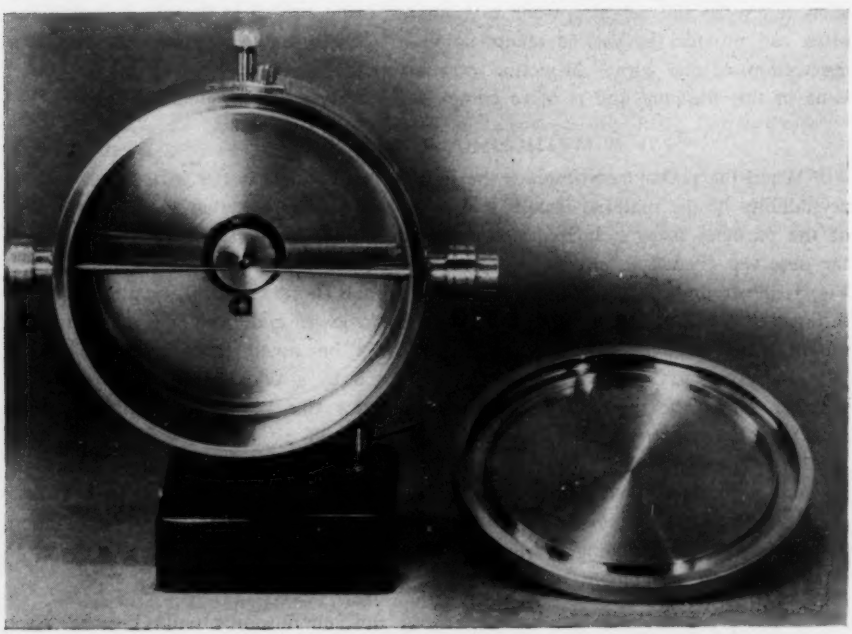

Figure 3

X-ray diffraction powder camera. Specimen is mounted in central chuck (a) in alignment with x-ray beam collimator (b) and beam trap (c).

of the 114.59 mm powder camera (Fig. 3), and adjusted so that it is bathed by the ½ mm beam of monochromatic x-radiation which proceeds from collimator (b) to beam trap (c). Nickel filtered copper K*a* radiation produced at 35 KV and 20 ma was used with the patterns shown in this paper. The exposure time varied from 2 to 4 hours.

REPORT OF CASES

Case 1. The patient was a 58 year old, white man, with a history of angina pectoris, who died following an acute myocardial infarction. At autopsy, the pericardium was thickened, yellow, firm and adherent to the heart by dense fibrous tissue bands (Fig. 4). Microscopic examination of this pericardial mass revealed a foreign body granuloma, containing many giant cells as well as many birefringent or double refractive crystals (Fig. 5). A small sliver of formalin-fixed tissue (washed as described above) was analysed by x-ray diffraction (Plate I, pattern A). This pattern was identical to the pattern obtained from Bakers U.S.P. talc (Plate I, Pattern B). Diagnosis: Talcum powder granuloma, pericardium.

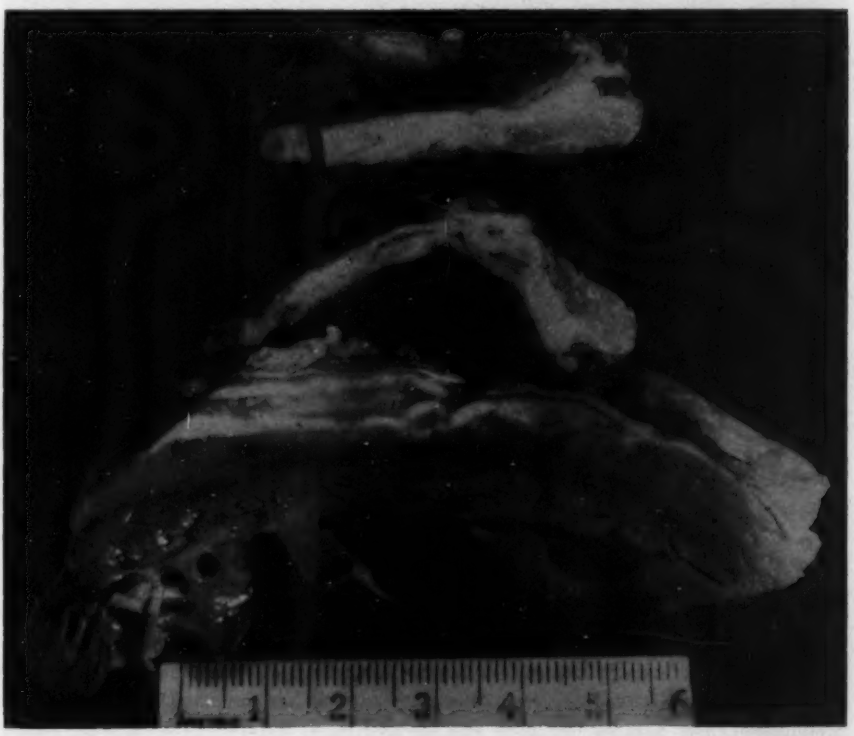

Figure 4

Gross section of fibrotic and adherent pericardium, talcum powder granuloma, Case 1.

Figure 5
Talc crystals in pericardium, Case 1, partially polarized, H and E stain, X430.

Case 2. This patient was a 43 year old white man with Hodgkin's disease who was treated with x-ray and nitrogen mustard. The patient died 5 years after the initial diagnosis. Autopsy revealed Hodgkin's disease, involving the spleen and lymph nodes. In addition, there was extensive calcification involving the heart, lungs, stomach, kidneys and spleen. The section of the heart, formalin-fixed, was analysed by x-ray diffraction. The resulting pattern (Plate I, Pattern C) was consistent with the pattern obtained from the complex calcium phosphate called apatite. The pattern for mineral apatite is shown in Plate I, Pattern D. When the individual crystallites involved are extremely small, as in the case of the body apatites, the diffraction pattern becomes relatively more diffuse, as can be seen by comparing pattern C with pattern D. Bones and teeth are also composed of apatite as a crystalline lattice.[3] Diagnosis: Calcification, heart (apatite).

Case 3. The patient was a 22 year old white man who developed 2 granulomatous lesions on the right arm (Fig. 6), coincident with the area where he had a tattoo two years prior to admission. The lesions seemed to cover only the red portions of the tattoo and had raised, indurated, purple margins with red granulation tissue centrally. No discharge was present and only moderate tenderness. The ulceration started about six months before admission. The lesion was excised and prepared for microscopic examination. The base of the ulcer was covered by a fibrinous exudate. The underlying granulation tissue extended deep into the subcutaneous fat. Plasma cells, lymphocytes and many eosinophiles were observed microscopically under oil im-

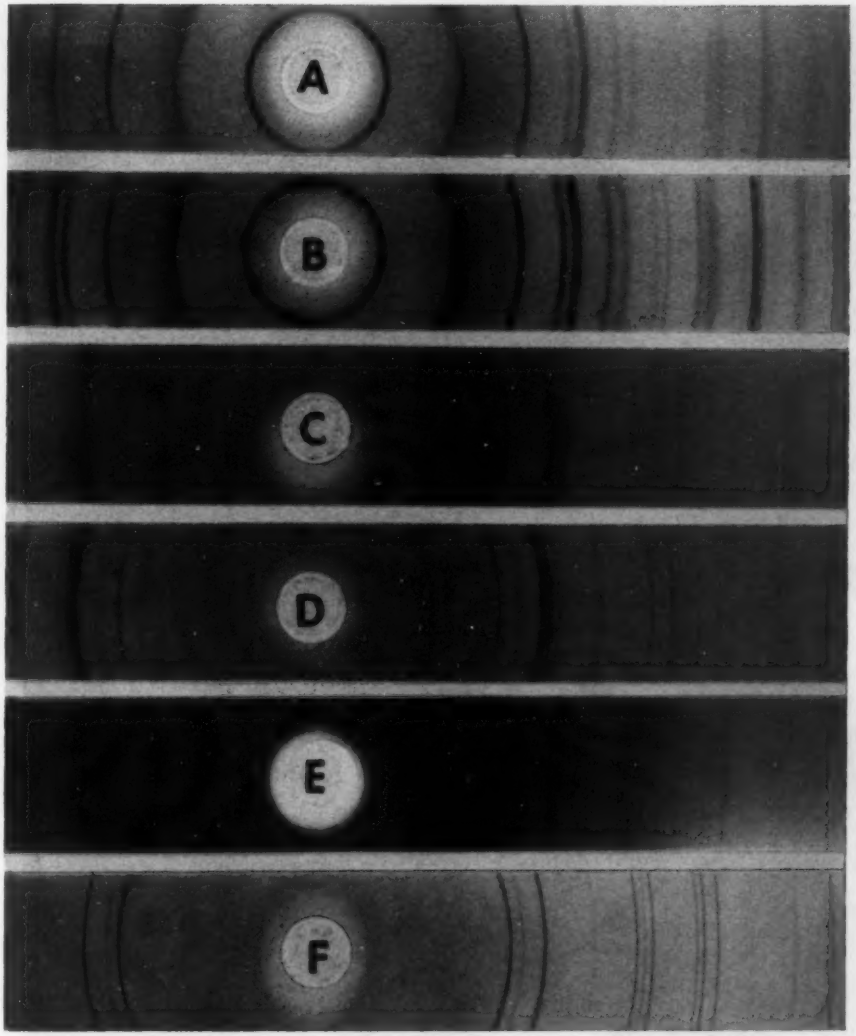

PLATE I

mersion. No giant cells were present. Small collections of reddish brown crystals were scattered throughout the tissue section (Fig. 7). A sample of formalin-fixed tissue from the ulcerated area was analysed by x-ray diffraction (Plate I, pattern E). In spite of the dark band present, due to the scattering by amorphous or non-crystalline tissue, the superimposed lines present in pattern E agree with the x-ray diffraction pattern from C.P. red mercuric sulfide (Merck) (Plate I, pattern F). Diagnosis: Tattoo dermatitis, probably secondary to mercuric sulfide (cinnabar).

Figure 6
Tattoo granuloma, Case 3.

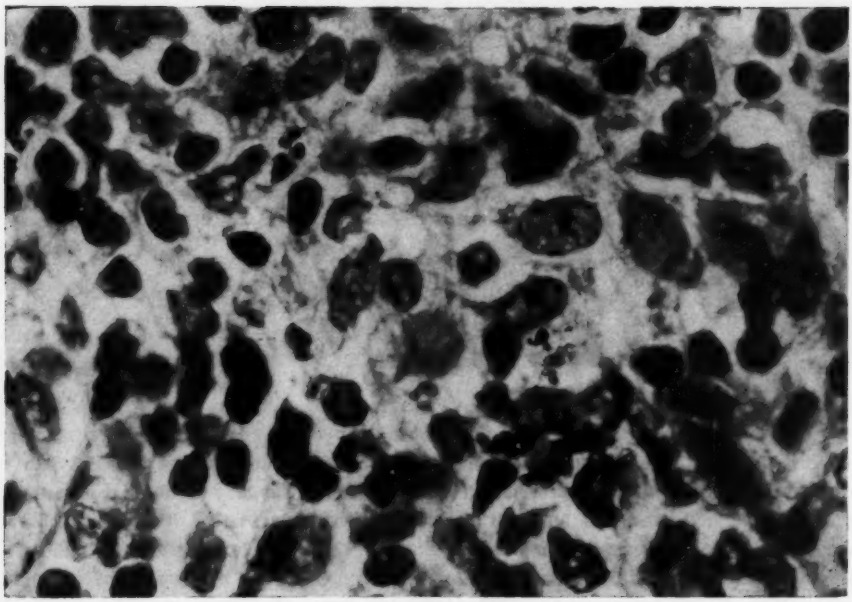

Figure 7
Mercuric sulfide crystals in tattoo granuloma, Case 3, H and E stain, X1100.

Case 4a. The patient was a 53 year old, white man, who died in renal failure, with a clinical diagnosis of collagen disease. He had been a pottery factory worker for 6 years, and had received injections of thorotrast 11 years prior to death as a diagnostic procedure for suspected brain tumor. Microscopic examination of the liver and spleen revealed scattered small deposits of brown crystalline material. In the liver (Fig. 8) these crystals were observed lying free in portal areas, around central veins, and in Kupffer cells. In the spleen the deposits were larger and more numerous than in the liver. Identical x-ray diffraction patterns were obtained from the liver (Plate II, pattern G) and spleen tissue specimens. Comparison of the latter pattern with one obtained from a known thorium dioxide preparation, (Heyden Chemical Corporation, New York) (Plate II, pattern H) shows close basic agreement. Diagnosis: Thorium dioxide deposits (Thorotrast)—liver and spleen.

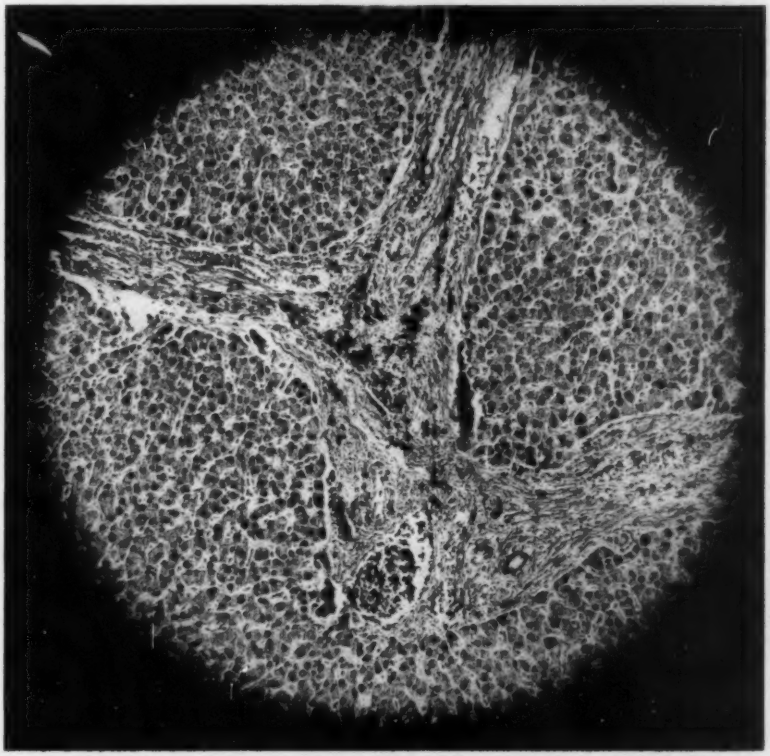

Figure 8

.Thorium dioxide deposits in liver, Case 4a, H and E stain, X225.

Case 4b. In the same case (Case 4a), the visceral pleural surfaces and lung parenchyma of all lobes of both lungs were studded with numerous tiny, firm, white nodules, averaging 1 to 5 mm in diameter. Microscopic examination of the nodules revealed dense whorls of acellular hyaline tissue, surrounded by a zone of proliferating

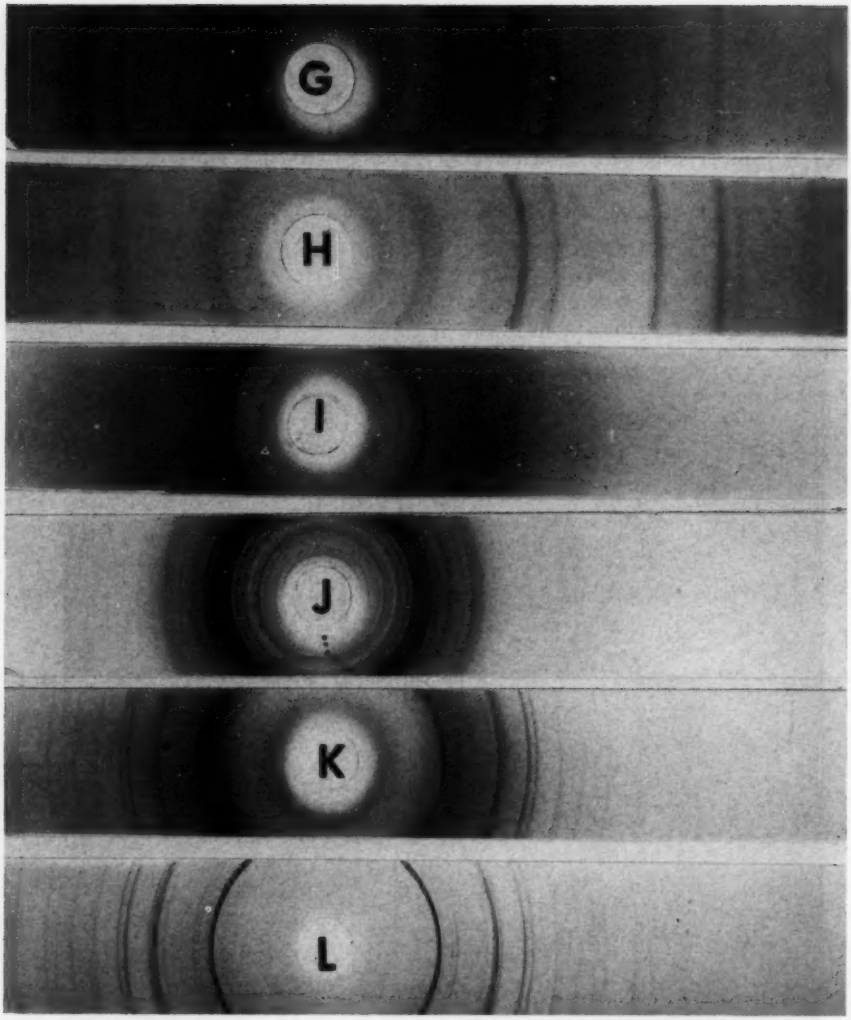

PLATE II

fibroblasts and numerous pigmented macrophages (Fig. 9). Scattered collections of anthrocotic pigment were noted in the central hyaline mass, together with deposits of basophilic material. In the polarizing microscope, crystals were seen both in and around the hyaline mass (Fig. 10). One of the nodules, formalin-fixed, was pressed into the glass capillary glass tube and analysed by x-ray diffraction. The resulting pattern (Plate III, pattern N) was observed to consist of lines characteristic of kaolin (Merck) (Plate III, pattern M) and quartz (A. D. Mackay, Inc.) (Plate III, pattern O). Final diagnosis: Pneumoconiosis, associated with silica (quartz) and kaolin.

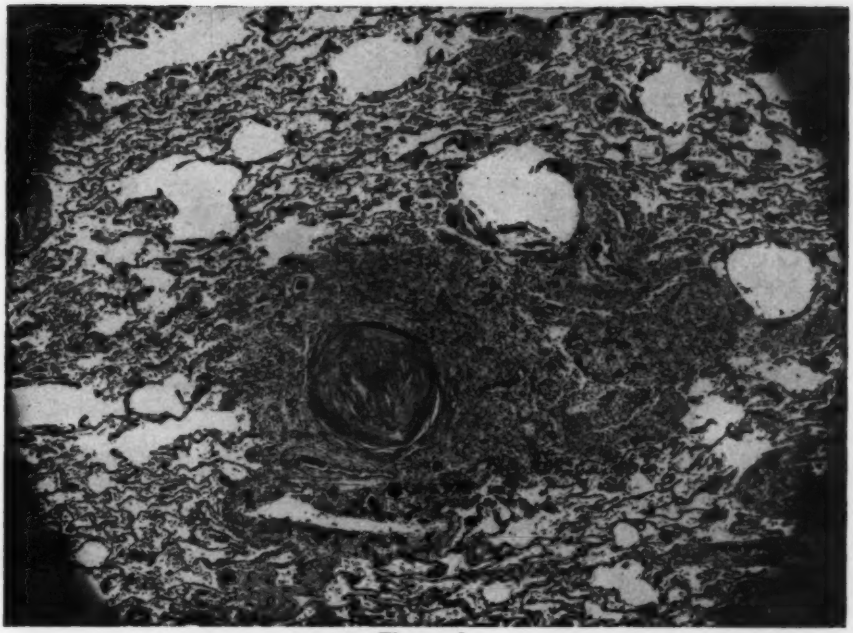

Figure 9

Silicotic lung nodule, Case 4b, H and E stain, X35.

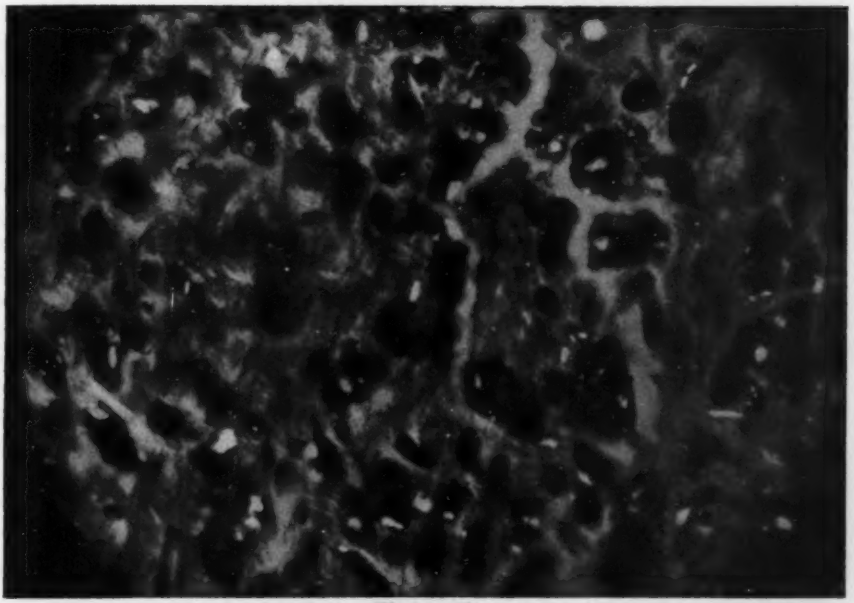

Figure 10

Crystals in lung nodule, Case 4b, in partially polarized light, H and E stain, X730.

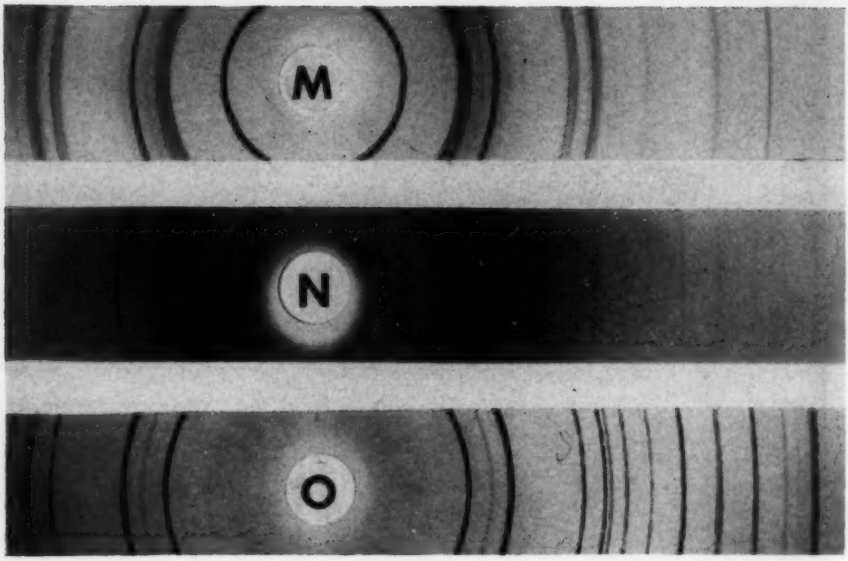

PLATE III

Case 5. The patient, a 4½ year old white female, was brought to the hospital because of severe headaches and vomiting occurring at intervals during the 4 months prior to admission. The general physical examination was normal. The neurological findings included an enlarged cranial vault, marked bilateral papilledema, a mild right facial weakness, and a broad-based gait. Routine laboratory studies were within normal limits. The cerebrospinal fluid protein was 110 mgms. percent. Skull x-rays revealed evidence of increased intra-cranial pressure, and a calcified supracellar mass. Under general anesthesia ventriculography was performed through the coronal sutures. The ventricles were markedly enlarged and under increased pressure. During the exchange of fluid and air, a cystic cavity was encountered and about 90 cc of a oily, xanthrochromic fluid was withdrawn and replaced with air. It was noted that numerous crystals were present in the later cystic fluid, which was sent to the Physics Department for x-ray diffraction analysis. The crystals were removed from the fluid by alternate washing and centrifuging (Fig. 11). Although under the polarizing microscope these crystals gave the appearance of cholesterol, they were definitely established as such by comparison of the x-ray diffraction patterns for the brain crystals and for standard cholesterol (Plate II, Patterns I and J). In this case a ventriculogram revealed an enlarged ventricular system and a huge suprachiasmatic cyst. Through a right frontal craniotomy a large craniopharyngioma was removed. Recovery was satisfactory.

Case 6. The patient was an 18 year old male who has been intermittently hospitalized since birth with the De Toni-Fanconi syndrome, a disorder of the kidney tubules secondary to cystine metabolism. A specimen of lymph nodes was obtained

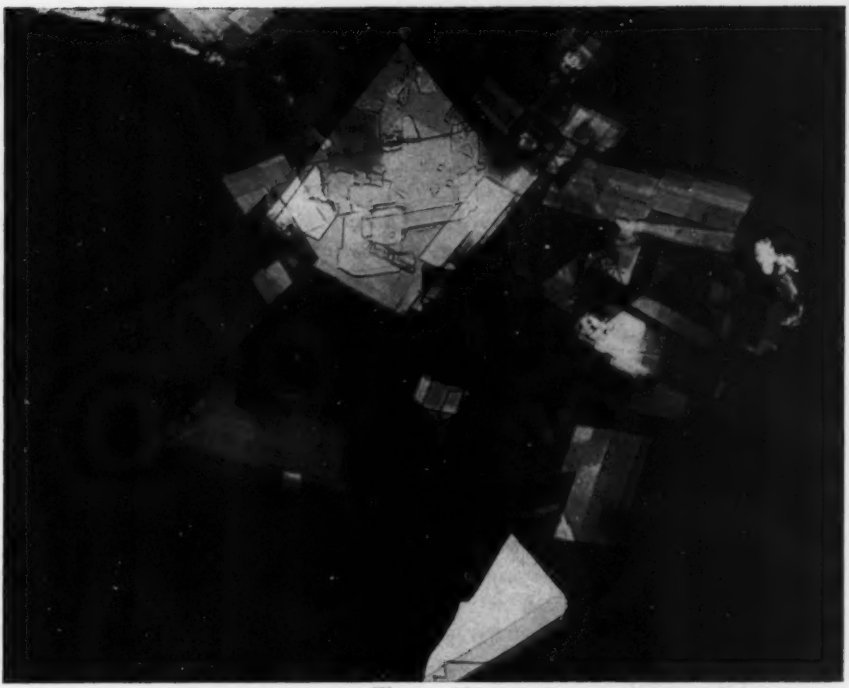

Figure 11

Crystals extracted from intra-cranial cyst fluid, Case 5, taken with partially polarized light, X115.

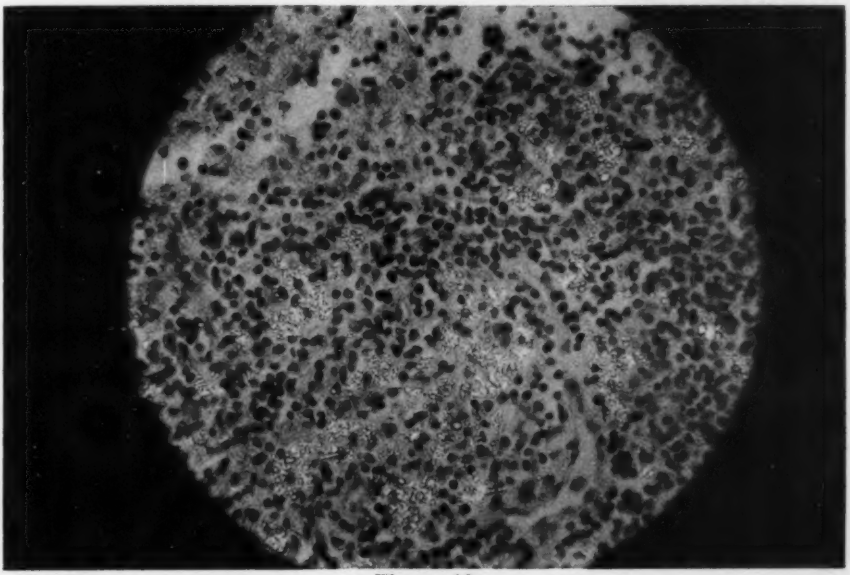

Figure 12

Cystine crystals in lymph node, Case 6, taken with partially polarized light, X600.

from biopsy and sent to the Physics Department for x-ray diffraction analysis when crystals were observed microscopically in partially polarized light (Fig. 12). The diffraction pattern from the lymph node tissue (Plate II,, Pattern K) matched the pattern from standard cystine (Plate II, Pattern L).

DISCUSSION

In illustrating the use of x-ray diffraction in the cases presented, it has been the intent of the author to show only the analysis, by direct visual comparison, of unknown and standard x-ray diffraction patterns. This is the fastest and usually sufficient method of pattern interpretation, when standard patterns of suspected materials are available. When this is not the case, the patterns may be measured and the data

Table I

CASE 4b

Kaolin Standard (Merck) Pattern M, Plate III		Lung Tissue Pattern N, Plate III		Silicon Dioxide (Quartz) Pattern O, Plate III	
d	I/I_1	d	I/I_1	d	I/I_1
7.20	1.00	7.14	0.49		
4.41	0.38	4.42	0.27		
		4.25	0.67	4.26	0.35
4.19	0.38				
3.86	0.15	3.87	0.27		
3.58	0.88	3.58	0.27		
3.39	0.11				
		3.34	1.00	3.34	1.00
3.13	0.08				
2.75	0.05				
2.57	0.28	2.56	0.20		
2.53	0.20				
2.49	0.28	2.45	0.09	2.46	0.12
2.39	0.11				
2.35	0.63	2.34	0.13		
2.30	0.28	2.27	0.13	2.28	0.12
				2.24	0.06
2.20	0.11				
		2.13	0.09	2.13	0.09
2.00	0.20	1.99	0.13	1.98	0.06
1.94	0.11				
1.90	0.15				
1.85	0.15	1.82	0.20	1.82	0.17
1.79	0.15				
1.69	0.11				
1.67	0.20	1.67	0.09	1.67	0.07
1.63	0.15			1.66	0.03
1.59	0.05				
1.54	0.11	1.54	0.13	1.54	0.15
1.49	0.38	1.49	0.09		
1.43	0.08	1.46	0.04	1.45	0.03
1.39	0.03			1.38	0.07
		1.37	0.09	1.37	0.11
1.34	0.11	1.34	0.02		

obtained compared to a published index of x-ray diffraction data.[4] The use of this method is fully described in a previous issue of this *Bulletin*.[1]

When two or more separate crystalline materials are present in an unknown specimen, their individual diffraction patterns appear superimposed upon each other in the unknown pattern. This condition is illustrated in Case 4b where both kaolin and silica (quartz) are present (Plate III, paterns A and C). In such cases it is usually necessary to measure the unknown pattern lines. This has been done for the latter case and the comparative data are presented in Table I. The columns marked "d", in the table, contain the interplanar spacing distances, measured in Angstrom units, for the principal atomic planes present in the crystalline lattice structures involved. The columns marked I/I_1, give the relative intensities of the diffraction lines to other lines in the same crystal pattern. The complete diffraction data for some of these cases, with other cases and data, has been previously published[5] with discussion and literature references to similar cases.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the help of the following individuals for contributing tissue specimens and case history information used in this paper: (1) Dr. Frank J. Eurs, Associate Pathologist, Women's Hospital, Detroit and former Resident in Pathology, Henry Ford Hospital; (2) Dr. Reuben Meyer, Children's Hospital, Detroit; (3) Dr. John R. Bartone, Neuro-Surgery Department, Henry Ford Hospital.

The technical assistance of Miss Dorothy Hebert is acknowledged with thanks.

REFERENCES

1. Parsons, J., Beher, W. T., and Baker, G. D.: X-ray diffraction powder data and index for the steroids, Henry Ford Hosp. M. Bull. 6:365, 1958.

2. Klug, H. P., and Alexander, L. E.: X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials, New York, Wiley, 1954.

3. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

4. American Society for Testing Materials: X-ray Powder Data File, Philadelphia, 1958.

5. Parsons, J., and Eurs, F. J.: X-ray analysis of crystals in pathology, Am. J. Clin. Path. 32:405, 1959.

ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

REVERSED FIGURE SIX ILEORECTOSTOMY. J. BARRON. Dis. Colon & Rectum 2:452, 1959.

This loop procedure has been employed in 10 cases of ulcerative colitis and five cases of multiple polyposis. In all but two instances, colectomy and anastomosis were performed in one operation. In two patients, the ileostomy was performed first and later the colon was removed and the ileum was attached to the rectum. All of the cases of ulcerative colitis represented the most serious form of the disease and had failed to respond to intensive and prolonged medical treatment. In these patients there was rectal involvement of some degree and all had moderate to severe toxemia. With increased experience, I believe we will have reason to feel quite encouraged and with the passage of time I believe we will find that the use of ileal loops will provide certain advantages not only at the time of surgery but in the postoperative period. The possibility of rectal preservation will always attract many patients and it should be possible for the surgeon to accomplish it in the earlier stages of the disease before it has advanced to the point where surgical intervention is hazardous or malignant degeneration has occurred.

***MECHANISM OF AMINONUCLEOSIDE-INDUCED NEPHROSIS IN THE RAT. I. METABOLISM OF TRITIATED AMINONUCLEOSIDE.** P. BARTLETT AND S. SHELATA. Proc. Soc. Exper. Biol. & Med. 102:499, 1959.

The preparation and metabolism of radiochemically pure aminonucleoside, 6-dimethylamino-9-(3'-amino-3'-deoxy-β-D-ribofuranosyl)-purine, labeled with tritium by the Wilzbach procedure, is described and discussed. Data are presented for the distribution of radioactivity in body fluids and urine as well as in kidney and liver nucleic acids. Study of the chemical nature of tritium activity in an acid-soluble nucleotide fraction, prepared from pooled kidney tissue of rats rendered nephrotic with the tritiated aminonucleoside, indicates that the rat is capable of metabolizing the latter into labeled products utilizable in the biosynthesis of adenosine and cytidine nucleotides. Labeling of these nucleotides, which cannot be accounted for in terms of an exchange mechanism involving the hydrogens of body water, is considered in terms of alternative metabolic pathways.

***EFFECT OF CHOLIC AND HYODEOXYCHOLIC ACIDS ON METABOLISM OF EXOGENOUS CHOLESTEROL IN MICE.** W. T. BEHER, G. D. BAKER, AND W. L. ANTHONY. Proc. Soc. Exper. Biol. & Med. 103:385, 1960.

In the female mouse fed a cholesterol-free diet: (1) supplement of 1% cholesterol did not alter liver or carcass cholesterol levels; (2) 1% cholesterol plus 0.5% cholic acid brought about large increases in liver and carcass cholesterol levels; (3) hyodeoxycholic acid reversed the effect of cholic acid and prevented cholesterol accumulation.

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*From Edsel B. Ford Institute For Medical Research.

*INFLUENCE OF BILE ACIDS ON CHOLESTEROL METABOLISM IN THE MOUSE. W. T. BEHER, W. L. ANTHONY, AND G. D. BAKER. Proc. Soc. Exper. Biol. & Med. 102:317, 1959.

Feeding various bile acids to mice for 3 weeks had the following effects: Cholic acid increased hepatic and intestinal cholesterol levels, but had no effect on kidney cholesterol. It decreased hepatic cholesterol synthesis rates, but had no effect on intestinal synthesis rates. Hyodeoxycholic and lithocholic acids decreased liver cholesterol levels. Hyodeoxycholic acid had no effect on intestine and kidney cholesterol, or on intestinal cholesterol-x-C^{14}. Both acids effected large increases in hepatic cholesterol synthesis. Deoxycholic acid significantly decreased liver, small intestine and kidney cholesterol levels. It also decreased hepatic cholesterol synthesis. Results of this study suggest that homeostatic control of cholesterol metabolism in tissues other than liver is independent of hepatic control.

*INHIBITION OF CHOLESTEROL BIOSYNTHESIS BY CHOLIC ACID. W. T. BEHER AND G. D. BAKER. Am. J. Physiol. 197:1339, 1959.

The effects of dietary cholic acid on free, total and ester cholesterol levels in liver and serum, and on the relative rates of incorporation of acetate-I-C^{14} and mevalonic acid-2-C^{14} were investigated in the rat. Cholic acid caused equally significant increases in free and ester lived cholesterol. No significant changes were found in serum cholesterol levels. The incorporation rate of acetate-I-C^{14} into liver and serum cholesterol was inhibited by 65% when cholic acid was fed to rats, while the inhibition of mevalonic acid-2-C^{14} incorporation was 25%. It is suggested that cholic acid inhibits cholesterol biosynthesis between mevalonic acid and cholesterol, or retards the entire series of reactions between acetate and cholesterol.

THE SIGNIFICANCE OF PLEURAL EFFUSION COMPLICATING OTHERWISE OPERABLE BRONCHOGENIC CARCINOMA. G. L. BRINKMAN. Dis. Chest 36:152, 1959.

Bronchogenic carcinoma complicated by pleural effusion which is not attributable to congestive heart failure or systemic disease, presents a difficult problem in management. It is apparent that the presence of pleural effusion, whether malignant cells are demonstrated in the fluid or not, is of serious prognostic significance. At thoracotomy, 17 of these patients showed evidence of extrapulmonary spread of their disease, and only one has survived for over two years. Twenty-one cases of bronchogenic carcinoma with pleural effusion occurring as the only manifestation of possible extrapulmonary involvement are reported. All were subjected to thoracotomy, but only survived for more than two years. It is questioned whether thoracotomy is justified in these patients, even though malignant cells are not demonstrated in the pleural fluid.

SOME DEVICES FOR THE MANAGEMENT OF LYMPHEDEMA OF THE EXTREMITIES. B. E. BRUSH, J. H. WYLIE, JR., AND J. BENINSON. S. Clin. North America 39:1493, 1959.

Lymphedema of the extremities is a common, distressing and disabling condition. In the leg, it may be the sequel of phlebitis, inguinal lymph node dissection, obstruction

*From Edsel B. Ford Institute For Medical Research.

of the veins for any reason, lymphangitis and cellulitis. The etiology of some swellings, as in Milroy's edema, is obscure. By far the most common cause of swelling of the arm is radical mastectomy, with and without postoperative irradiation. We have found that several new mechanical devices are of great value in the treatment of swelling of the leg or arm. There are improved models of apparatus for the application of intermittent pressure as a primary part of the therapy. There are available especially efficient types of elastic stocking, leotard and sleeve, which are custom-made for the individual patient. A total of 275 patients have been treated by intermittent positive pressure and the JOBST elastic stocking or sleeve. There were 160 upper extremities and 115 lower extremities. The results in most of these have been gratifying, especially when treatment has been vigorous and prolonged. There have been no significant complications of treatment. Recurrent lymphangitis has been almost nonexistent. Only rarely has a patient objected to the inconvenience of the elastic sleeves or stockings.

SOME BASIC ASPECTS OF CLOT DISSOLUTION. M. J. CALDWELL, R. C. HORN, JR., AND S. A. JOHNSON. Am. J. M. Tech. 25:373, 1959.

From a survey of the literature it is apparent that intravascular clotting is being studied from two diverse approaches, from that of clot formation on the one hand, clot dissolution on the other. Both lines of investigation are very complex, but it appears now that more attention is being paid to the problems, and the ability to use some of the simple fibrinolytic tests is becoming important to the laboratory technologist. When a fibrinolytic test is being used to follow intravenous infusion of the active enzyme, fibrinolysin, it is necessary to take great care with the blood sample. The active enzyme, as such, is very labile and the assay must be carried out as soon as the blood is drawn or the plasma must be frozen immediately. The interference of fibrinolytic activity in the determination of fibrinogen is emphasized.

COMBINED OPERATION OF GASTRECTOMY AND VAGOTOMY. L. S. FALLIS AND J. BARRON. West. J. Surg., Obst. & Gynec. 67:260, 1959.

Recurrent ulceration is the most serious of the postgastrectomy sequelae. The disappointment of the patient, caused by the reappearance of ulcer symptoms after an operation of the magnitude of subtotal gastrectomy, is matched only by the chagrin of the surgeon. Recurrent ulceration is rare after gastrectomy for gastric ulcer, but occurs with sufficient frequency after operation for duodenal ulcer to constitute a real problem. The addition of vagotomy to partial gastrectomy is an effective means of reducing recurrent ulceration. On the basis of this study postoperative ulceration appears to be commoner after the Billroth I than the Billroth II operation, even with the benefit of protective vagotomy. Recurrent ulceration after the Billroth I operation is usually controlled by medical measures, whereas when it occurs after the Billroth II procedure, reoperation is usually required.

THE WORKSHOP OF THE SURGEON — THE MODERN OPERATING ROOM. L. S. FALLIS, C. R. LAM, AND J. L. PONKA. S. Clin. North America 39:1469, 1959.

A description has been given of some of the things which the modern operating room provides which enhance the surgeon's ability to work and contribute to his comfort. Among these are convenient lights, easily maneuverable operating tables, a lounge and chapels for relatives, and comfortable shoes.

THE PUGH NAIL IN THE TREATMENT OF HIP FRACTURES. J. L. FLEMING. S. Clin. North America 39:1507, 1959.

The Pugh nail has been used in our hospital in 39 cases within the past 28 months. These were all intracapsular fractures including basilar, neck and subcapital. One pathological fracture was included. Early failure occurred in two instances as a result of poor placement of nail in one and poor reduction in the second. In one case the nail backed out at three weeks and renailing was necessary. Aseptic necrosis was a complication in this case. With this exception nonunion has not been a factor in our cases, but in many it is too early for a final statement. The Pugh nail is a distinct advance in treatment of "the unsolved fracture" and its precise use is recommended to the surgeon operating upon hip fractures.

SIMULTANEOUS HYPERTHYROIDISM AND HYPERPARATHYROIDISM. B. FRAME AND R. H. DURHAM. Am. J. Med. 27:824, 1959.

A case of hyperthyroidism and hyperparathyroidism occurring simultaneously is reported. The presence of a kidney stone and peptic ulcer, present before the onset of symptoms of hypermetabolism, suggests that the hyperparathyroidism preceded the hyperthyroidism.

PULMONARY RADIATION REACTION: A VITAL-CAPACITY AND TIME-DOSE STUDY. J. R. GISH, E. O. COATES, L. A. DUSAULT, AND H. P. DOUB. Radiology 73:679, 1959.

A study has been made of the pulmonary radiation reaction with reference to the time-dose relationship. Vital capacity studies were made on patients who showed pulmonary reaction. The vital capacity studies showed mild changes from the normal. None of these patients exhibited clinical signs of impaired pulmonary function. In patients who received 250-kv roentgen therapy, 44 per cent showed some evidence of pleuropulmonary reactions. In those receiving cobalt-60 therapy, only 20 per cent showed pleuropulmonary reactions, although they received a higher tissue dose. The small degree of lung reaction is largely attributable to tangential therapy and modern methods of lung-shielding in routine cases.

*EFFECT OF PARTIAL COAGULATION UPON THE SIZE DISTRIBUTION CURVE IN HETERODISPERSE COLLOIDAL SYSTEMS. W. HELLER, E. ROWE, R. BERG, AND J. H. D. WATSON. J. Phys. Chem. 63:1566, 1959.

The size distribution of the unaggregated particles remaining after partial coagulation of a colloidal solution of spheres is found to be altered in favor of the large particles as compared to the distribution in uncoagulated control specimens. The results obtained in a di-disperse latex of polystyrene were quantitatively compared with the ones expected on the basis of Müller's theory. The order of magnitude of the effect observed agrees with that expected from the difference in probability of collisions between unequal sized particles as compared to equal sized particles. The same type of shift is found for a heterodisperse polystyrene latex. All results can be explained satisfactorily without the assumption that the collision number (the "stability") varies with particle size within the colloidal range.

*From Edsel B. Ford Institute For Medical Research.

DENTAL CARE PLANNING IN HOSPITALS. F. A. HENNY. J. Oral Surg., Anesth. & Hosp. D. Serv. 17:18, 1959.

A well staffed, well equipped dental department contributes much to the over-all care of both resident and ambulatory hospital patients. The hospital is also the proper atmosphere for the dental care of the physically and mentally handicapped. In addition, hospitals provide excellent facilities for formal graduate education in the dental specialties and should also function as centers for continuing education of dentists at the community level.

PRESENT AND FUTURE PROBLEMS IN ORAL SURGERY. F. A. HENNY. J. Oral Surg., Anesth. & Hosp. D. Serv. 17:66, 1959.

Although the specialty of oral surgery has made great progress in recent years, many problems remain. The solution to them is largely dependent on the constant improvement of graduate education and the continuing development of a strong national oral surgery organization. Both will contribute greatly to an ever-increasing quality of patient care.

ROLE OF THE ELECTROCARDIOGRAM IN THE DIFFERENTIAL DIAGNOSIS OF ACUTE CHEST PAIN. T. N. JAMES. South. M. J. 52:1391, 1959.

In this review discussion was limited to electrocardiogram features of diseases that may cause acute pain in the chest. In some of these diseases other laboratory examinations are more important than electrocardiography, and in all of them a good clinical examination is the most valuable. By knowing what may be expected of the electrocardiograph, however, one may obtain the utmost value of that instrument. The pertinent positive and negative electrocardiographic findings that occur in certain diseases that cause acute chest pain have been described. The diseases considered include angina pectoris, myocardial infarction, pulmonary embolism, pericarditis, dissecting aneurysm and spontaneous pneumothorax.

*FUNCTIONAL ORGANIZATION OF THE RESPIRATORY CHAIN IN LIVER MITOCHONDRIA. T. KIMURA AND T. P. SINGER. Nature 184:791, 1959.

The question of whether the mitochondrion is compartmentalized into separate respiratory chains serving the individual cytochrome-linked dehydrogenases and acting independently of each other, or whether all or most of these dehydrogenases are structurally and functionally linked to a common electron transport system, has been an open and much debated one. The findings suggest that the respiratory chains of liver mitochondria, at least so far as the succinic and choline oxidase systems are concerned, are not compartmentalized, but are interlinked at and above the oxidation-level of cytochrome c_1, although the *b* components do not appear to be on a common path. Intercommunication between the chains (alternative (3)) is indicated by the fact that the equantitative effect of titration with an inhibitor, such as azide or cyanide, depends on the relative turnover rates of the dehydrogenase and the cytochrome component being titrated, respectively, and may be altered by depressing the activity of the dehydrogenase. Neither mechanism (1) nor (2) is compatible with this behaviour, but it is to be expected from the third one.

*From Edsel B. Ford Institute For Medical Research.

SOME ADDITIONS TO AND SUBTRACTIONS FROM THE ARMAMENTARIUM OF THE CARDIAC SURGEON. C. R. LAM. S. Clin. North America 39:1553, 1959.

This article contains much that is historical. The instruments used for the first operation on the mitral valve in 1925 by Souttar are shown. Twenty years later there had been no important additions to the armamentarium of the cardiac surgeon except for the modifications in artery clamps made by Gross and by Crafoord for their operations for patent ductus arteriosus and coarctation of the aorta. Then came the clamps for the "blue baby operations," the Blalock clamp for the subclavian-pulmonary anastomosis and the Potts-Smith clamp for the side-to-side aortic-pulmonary anastomosis. Experiences with the six-fingered glove for mitral commissurotomy, the two-pointed needle in atrioseptopexy and of the evolution of pulmonary valvotomy are discussed.

FRACTURES AND FRACTURE — DISLOCATIONS OF THE ANKLE. C. L. MITCHELL AND J. L. FLEMING. Postgrad. Med. 26:773, 1959.

Accurate diagnosis and proper choice of treatment in fractures and fracture-dislocations of the ankle require a knowledge of the mechanisms of injury. Good x-rays, centered over the joint in true anteroposterior and lateral projections, are also prerequisites to accurte diagnosis. Closed methods of treatment may be used in many cases, but certain fractures require open reduction. Surgery must be precise, and, to allow the indicated correction to be made, x-rays should be taken in the operating room before the wound is closed.

PRESIDENTIAL ADDRESS (AMERICAN ORTHOPAEDIC ASSOCIATION, 1959) C. L. MITCHELL. J. Bone & Joint Surg. 41-A:1157, 1959.

Great changes have taken place in the past twenty-five years in the practice of orthopaedic surgery and in the training of our residents. Unquestionably great advances have been made in the training programs, the clinical approach to orthopaedic problems, and in conducting and stimulating basic and clinical research in our specialty. However, although great advances in the progress of orthopaedic surgery have been made in the past and will continue to be made in the future by individuals of stature in our profession working more or less independently, we must recognize the importance of the team approach to problems of mutual interest in research, clinical investigation, and therapy. We must accept and assume our rightful role as leaders in the over-all care of patients with problems primarily of an orthopaedic nature but be prepared to cooperate to the fullest with other medical specialties involved. We should forever strive to take advantage of knowledge and assistance from other medical or scientific sources for conducting of research projects and for improvement in the clinical treatment of our patients. In our training programs we should provide an opportunity for our residents to grasp a firm knowledge of the sociomedical and medical-economic aspects of our specialty and instill in them the idea of the team approach and the total care of the patient.

CARDIAC ARREST OUTSIDE THE OPERATING ROOM WITH SUCCESSFUL RESUSCITATION: REPORT OF TWO CASES. C. MOTA, E. A. ZIKRIA, AND R. E. TABER. Ann. Int. Med. 51:577, 1959.

A review of the literature concerning successful resuscitation in cardiac arrest occurring outside the operating room reveals 13 cases. Two additional cases who

have made a complete recovery are herein reported. A description of the cardiac resuscitation method employed is outlined. In the future there should be more instances of successful resuscitation of patients dying suddenly from coronary artery disease. Two prime indications for the use of manual cardiac systole are demonstrated by the cases we describe. One ambulatory patient had had known coronary artery insufficiency and died suddenly, the other was under treatment for a myocardial infarction at the time of sudden and unexpected death.

EFFECT OF MACROGLOBULINS AND THEIR DISSOCIATION UNITS ON RELEASE OF PLATELET FACTOR 3. M. R. PACHTER, S. A. JOHNSON, AND D. H. BASINSKI. Thrombosis et Diathesis Haemorrhagica 3:501, 1959.

Recent work has shown that macroglobulins prepared from patients afflicted with macroglobulinemia of Waldenström have the capacity to "coat" the platelet surface and prevent the normal release of the prothrombin activation component, platelet factor 3. On the basis of coagulation, fluorescence and electron microscopical studies the authors proposed that the hemorrhagic diathesis, noted in some two thirds of all cases of primary macroglobulinemia, might frequently be accounted for on the basis of this mechanical blockade. The possibility of a combination of an external effect plus entrance of the macroglobulin into the platelet with resultant deleterious action on the contained elements has also been postulated. Despite the ability to abolish the "coating phenomenon" by use of specific rabbit-anti-macroglobulin serum the exact relationship that exists between the macromolecule and the platelet surface has remained obscure. It has been shown that dissociation units of native macroglobulins fail to exert any deleterious effect upon platelets. Evidence is presented to support the theory that molecular size is of paramount importance in preventing platelet factor 3 release.

MACROGLOBULINEMIA OF WALDENSTRÖM: CLINICAL, LABORATORY AND ANATOMIC FEATURES. M. R. PACHTER. Postgrad. Med. 26:315, 1959.

In macroglobulinemia of Waldenström, there appears to be a questionably malignant involvement of the lymphatic reticuloendothelial system which causes bleeding, anemia and infections. The etiology is unknown and the outcome is eventually fatal. Various clinical features and laboratory findings help to differentiate this disease from multiple myeloma, chronic lymphatic leukemia, lymphosarcoma and purpura hyperglobulinemia. Treatment is symptomatic, consisting of blood tranfusions and administration of adrenocortical steroids and adrenocorticotropic hormones.

COEXISTING CARCINOMA AND DIVERTICULA OF THE COLON; A REVIEW OF THREE HUNDRED FIFTY-FIVE CASES OF CARCINOMA OF THE COLON. J. L. PONKA, J. D. FOX, AND B. E. BRUSH. A.M.A. Arch. Surg. 79:373, 1959.

A review has been made of 355 cases of carcinoma of the colon occurring in the five-year period 1953-1957 inclusive. The location of these lesions and percentages has been noted. In 75 cases (21%) diverticulosis and diverticulitis were present as well. A close study was made of the 35 cases of sigmoid carcinoma with closely associated sigmoid diverticula. The clinical suspicion of carcinoma was the most valuable single diagnostic aid noted. Proctosigmoidoscopic examination was directly valuable in diagnosing carcinoma and confirmed the clinical impression of carcinoma in 57% of our cases. It was of little value in diverticulosis. The surgical attitude of aggressive,

prompt action in all cases of diverticula which appear at all suspicious of carcinoma is the one method by which cure rates may be improved. Surgery should be used more frequently in carefully selected cases. Such an aggressive attitude will undoubtedly save lives in the future, in a population which is aging and destined to have more and more colonic diseases.

CYST OF THE LEFT TRIANGULAR LIGAMENT OF THE LIVER. A. K. POZNANSKI. Radiology 73:896, 1959.

Cysts of the hepatic ligaments are relatively rare as shown by the fact that only 7 cases could be found in the literature. Four of these were in the falciform ligament and 3 in the ligamentum teres. No cases were described in the triangular ligaments. A case of cyst of the triangular ligament of the liver is presented, which was best demonstrated by the aid of pneumoperitoneum.

FAMILIAL RECURRING POLYSEROSITIS: A DISEASE ENTITY. R. J. PRIEST AND R. K. NIXON. Ann. Int. Med. 51:1253, 1959.

The clinical and diagnostic criteria of familial recurring polyserositis have been presented. It is likely to be another disease added to the list of inborn errors of metabolism. Its etiology and pothogenesis are incompletely understood, although its hereditary nature has been established in certain ethnic groups. Patients with this disease face serious economic and sociologic problems because of the seemingly interminable recurrences. The recognition of this entity must be made more widespread, and it is hoped that the grim prospect of its chronic recurrence will be lightened by further research.

THE MANAGEMENT OF GASTRIC POLYPS. T. BRIDWELL AND R. J. PRIEST. Bull. Gastroscopy & Esophagoscopy 6:1959.

Eighty patients with gastric polyps have been studied from a group of 3,000 gastroscopies (2.4 percent). The factors influencing the decision for management have been evaluated, including initial and progress x-ray and gastroscopic examinations and other clinical and laboratory criteria. Small gastric polyps, benign in appearance, can be followed by progress x-ray and gastroscopic observation. One polyp with an intraepithelial carcinoma was encountered and one patient with adenocarcinoma had an associated benign polyp. It would appear from our experience that partial gastrectomy has been done more frequently than necessary. Gastrotomy with local excision of benign appearing gastric polyps has been made for surgical management rather than a medical followup.

ANESTHESIA: HISTORY'S ANSWER TO MAN'S NEED. M. REVZIN. J. Oral Surg., Anesth. & Hosp. D. Serv. 17:49, 1959.

Anesthesia developed in response to man's need to be free from pain due to illness and surgery. Its discovery and growth occurred when man's environment was ripe for its development. This article traces anesthesia from a superstition to a science.

USE OF RELAXIN IN MANAGEMENT OF ULCERATION AND GANGRENE DUE TO COLLAGEN DISEASE. H. REYNOLDS, AND C. S. LIVINGOOD. A.M.A. Arch. Dermat. 80:407, 1959.

The use of relaxin in the management of two patients with disabling trophic ulceration and gangrene, with Raynaud's phenomenon, due in one case to scleroderma-

dermatomyositis and in the other to scleroderma, is described. Significant improvement was obtained.

EVALUATION OF GELFILM® IN CYCLODIALYSIS CLEFTS — PART II. R. A. SCHIMEK AND D. BARSKY. Eye, Ear, Nose & Throat Month. 38:847, 1959.

Three patients with apparently intractable glaucoma were treated by cyclodialysis, with Gelfilm® inserts to hold open the cyclodialysis clefts. In only one patient was the tension lowered for a significant length of time; and this eye was finally enucleated in phthisis. With Gelfilm® inserts there was evidence of a minimal but prolonged intraocular inflammatory response, and there was formation of anterior peripheral synechiae to either side of the Gelfilm® inserts. Therefore, the use of Gelfilm® implants in cyclodialysis clefts is not recommended at the present time.

AN ELASTIC DACRON ARTERIAL SUBSTITUTE. D. E. SZILAGYI. S. Clin. North America 39:1523, 1959.

An account is given of the experimental and clincal findings in the use of an arterial substitute, woven of Dacron yarn, which is seamless, smooth-walled, finely porous, light, and has elastic qualities. In animal experiments, during 24 months of observation, the prosthesis showed low tissue reactivity, good arteriogenesis and excellent durability. Used as direct replacement and as by-pass graft in the aorta-iliac and femoropopliteal areas, in 186 clinical cases (the earliest of which has been followed for 24 months) the prosthesis has yielded patency rates only slightly lower than those obtained by us with homografts. The assumption that the *late* patency rate of these prostheses will be better than that of homografts is supported by both theoretical considerations and by experimental evidence, but it will not be proved or disproved until the results will have been followed for much longer periods of time.

CLINICAL CONTRIBUTION OF ELECTROCARDIOGRAPHY IN MECHANICAL MALFORMATIONS OF THE CARDIOVASCULAR SYSTEM. R. F. ZIEGLER. Am. Heart J. 58:504, 1959.

The principal contribution of the electrocardiogram in the study of patients with congenital cardiovascular malformations (most commonly mechanical defects with an otherwise normal myocardium) is the evidence it provides of single-chamber functional "strain" and anatomic "enlargement." Now it is usually considered that a cardiovascular examination is incomplete if any of the "standard," and perhaps some of the not so standard, technical diagnostic methods, such as electrocardiography, fluoroscopy and conventional radiography, cardiac catheterization, and contrast angiocardiography, are omitted. Which method provides a given type of diagnostic information with the least risk, the greatest technical ease and facility, the maximum accuracy, etc.? At least potentially, and to an already great extent actually, a properly recorded and accurately interpreted electrocardiogram probably provides the most specific clinical index to not only the presence but also the basic hemodynamic type, and to a certain extent the degree, of increased single or combined cardiac chamber work and its resulting anatomic enlargement. Specific electrocardiographic criteria are summarized briefly and illustrated as to simple basic patterns.

HENRY FORD HOSPITAL MEDICAL BULLETIN

ORTHOPEDIC ISSUE

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AUTHOR'S FOREWORD

All segments of the medical profession give much lip service to the need for basic research. Investigators seeking support all too often find that the words, which sounded so grand at the annual meeting, carry no backing in dollars or in administrative help. The vision of the speaker runs out at this time.

It is widely proclaimed that orthopaedic surgery needs basic research. It does, but it is difficult for a would-be investigator to find backing for an orthopaedic problem. If he has already found a cure for cancer, funds are forthcoming in unlimited amounts. When he was beginning to seek the answer to cancer, funds seemed to be the equivalent of water in the Sahara at noon. There are many sides to this problem, and efforts are being made to correct this situation so that the drought may be minimized in time and degree.

The writer has sampled the difficulties alluded to so briefly above.* Coming to Henry Ford Hospital was equivalent to arriving at an oasis! Relatively little was said about supporting research; instead a laboratory was built, equipped and manned and necessary channels of communication established to make it a going concern. The writer was then handed this set-up, freely and without any interference, in spite of the fact that the time devoted to research must of necessity hamper the clinical work that is not only necessary but is the life-blood of the institution. Seldom has the kind of vision illustrated by these briefly stated facts been encountered in medicine. It is an enormous tribute to the policy-making group of the Hospital, and in particular to Dr. C. Leslie Mitchell, that a basic program of research was encouraged in this fashion. The work that has been, is and shall be published from the Henry Ford Hospital Orthopaedic Research Laboratory literally is the result of Dr. Mitchell's solid aid. Without his aid, the writer would still be doing research but *would not have accomplished anything.*

Therefore, while the writer feels singularly honored to have an issue of the Bulletin devoted to his work, the reader should understand that it should be far better considered as Dr. Mitchell's issue.

Accordingly, the issue and the work published in it are dedicated to Dr. Mitchell. He has given solid evidence of his belief that research in orthopaedics should be done and supported. It is the job of those of us working in the Laboratory to justify his efforts.

HAROLD M. FROST, M.D.

*It should be noted here that there were bright spots before the current program at Henry Ford Hospital got underway. Help and encouragement were received from Dr. J. D. Godfrey and K. L. Terplan, Buffalo, N. Y., from C. O. Bechtol, Los Angeles, Calif., and from Dr. D. M. Bosworth, New York City.

EDITORIAL

The internal structure of bone and the factors influencing it have presented intriguing and challenging problems for many years. Even today the exact chemical composition of bone is not known. Men such as Haller, Havers, Goodsir, Ollier, McEwen, Hunter, and countless others have shed much light on the problems of bone structure and bone physiology. In the past half century, more attention has been paid to the role of bone as a mineral reservoir and the important part that it plays in the equilibrium of calcium and phosphorus metabolism. Many factors are still not clearly understood in the interrelationship of bone metabolism to various metabolic and endocrine disturbances. A careful study of the bone structure itself in normal and abnormal states would seem to be of paramount importance in the interpretation of various anabolic and catabolic bone conditions. Dr. Frost has been interested for some years in the study of microscopic bone structure, utilizing a method personally developed of studying fresh undecalcified bone. As a result of his study of countless numbers of normal and abnormal bones using special stain techniques, he has been able to develop criteria differentiating such bone conditions as osteoporosis and osteomalacia, and has noted other peculiarities of bone not hitherto recognized, such as "feathering," "micropetrosis," and early fatigue fractures. Much of this material has already been published.

The present edition of the Bulletin is devoted to a series of additional papers on the subject by Dr. Frost. These papers include studies pertinent to the following fields:

1. The mechanism of function of the skeleton as a depot for calcium phosphorus, other electrolytes, and buffers.
2. Measurement of osteoblastic activity in human bone.
3. Measurement of osteoclastic activity in human bone.
4. Further studies on bone feathering and bone structure.
5. Changes in bone affecting its role as a structural material.

Dr. Frost's fertile and inquiring mind has been challenging many of our previous concepts of bone structure and metabolism, and although he is free to admit that many of his findings are as yet inexplicable, it is unquestionably true that many of his facts are incontrovertible and far reaching in importance. Many of the studies are yet in their infancy and each new finding opens up new avenues of research. It might well be prophesied that as a result of Dr. Frost's work on this subject, we will have to change many of our concepts of bone metabolism and physiology, and in addition the clinical application of his findings will be of immeasurable value to the patient and to the physician.

C. Leslie Mitchell, M. D.

AN ECONOMICAL MICROFLUORESCENCE SET-UP FOR DETECTION OF TETRACYCLINES IN BONE

H. M. Frost, M.D.*

ABSTRACT

Wratten 47 and 47b filters between microscope and light source and Wr. 8 and 9 between eye and eyepiece provide a simple, effective means of detecting tetracycline fluorescence which is economical.

* * * * * *

The observation of Milch, Rall and Tobie[5,6] that tetracyclines become fixed in mineralizing bone and cartilage and can be demonstrated by fluorescence microscopy has opened the door for extensive in vivo investigation of bone physiology in man. Work with tetracyclines in this laboratory was stimulated by their papers. The cost of their fluorescent equipment was an obstacle however. A fluorescent set-up used by the writer since 1953 was adapted to the present problem and has the advantages of simplicity and economy. Originality is not claimed since the technique is in sporadic use in this country although unpublished.

MATERIAL

Any tissue containing tetracycline may be examined with the following technique. Undecalcified bone sections are best made by the writer's technique.[2] Soft tissues must be cut frozen. Acid pH must be avoided. Prolonged exposure of material to alcohol or water gradually removes the tetracycline. Permanent mounts are best made in HSR (Harleco), in which no fading has occurred in 5 years.

TECHNIQUE

Wratten 47 and 47B filters are placed in series between light source and substage condenser. Wratten 8 and 9 filters are placed in series over the eyepiece. The filters are available from Eastman Kodak as 2 inch square gelatin films for $0.50 each. Kohler illumination must be used. The light source should be a high intensity tungsten filament source; a 200 watt or more 35 mm slide projector is excellent. The condenser should be opened to maximum aperture after focusing; oiling it to the slide improves illumination considerably. A monocular tube provides the brightest image.

Objectives used should have as large N.A. and small magnification as can be found and used with eyepieces of 5X-8X. Apochromats are ideal.

The individual wishing to do fluorescence microscopy but who does not understand some of the above terms is encouraged to read the references listed.[1,3,4,7] Improper adjustment of microscope optics will cause any fluorescent equipment to behave poorly.

With the above set-up tetracycline labelled bone fluoresces a strong yellow to orange color on a faint magenta background, the latter caused by some leakage in the filters. The bone itself fluoresces a faint blue. Since the energizing band of the above set-up is at 430 mu in the visible blue no special glass is necessary in the optics. Fluorescence is detectable when the drug is present in less than microgram amounts.

*Associate Surgeon, Department of Orthopedics.

Strong fluorescence of bone labelled in vivo by drug dosages of 5 mg/kg of organism appears. Fluorescence of tetracyclines in other tissues and of other fluors may be observed with the above equipment.

REFERENCES

1. Allen, R. M.: The Microscope, New York, D. Van Nostrand, 1940.

2. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

3. Hardy, A. C., and Perrin, F. H.: Principles of Optics, New York, McGraw-Hill, 1932.

4. Jenkins, F. A., and White, H. E.: Fundamentals of Optics, ed. 2, New York, McGraw-Hill, 1950.

5. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

6. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of the tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

7. Richards, O. W.: Fluorescence microscopy. In, Mellors, R. C., ed.: Analytical Cytology: Methods for Studying Cellular Form and Function, New York, Blakiston, 1955, Chap. 5.

OBSERVATIONS ON FIBROUS AND LAMELLAR BONE*

HAROLD M. FROST, M.D.

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INTRODUCTION

Pathologists routinely observe fibrous and lamellar bone.[a] Most pathologists instinctively learn to recognize the difference between the two kinds and often also learn the significance of fibrous bone in microscopic sections of skeletal material. Since no coherent literature deals with the subject[1,2,5,7,8], the student of bone pathology may remain long unaware of the existence and significance of the only two fundamentally different types of bone found in the skeleton. Recognition of the two types is essential for proper interpretation of osseous pathology.

In this paper some of the differences between fibrous and lamellar bone are outlined. The significance of the two in microscopic sections will be indicated.[4] The presentation is begun with a consideration of the "building blocks" of bone in order to orient the reader and establish the meaning of some terms.

THE CONSTITUENTS OF BONE[3,6,8]

Both fibrous and lamellar bone are composed of 4 major fractions.

The first fraction is the mineral moiety. This is composed of hydroxyapatite crystals which are about 300 Angstroms long and are composed of Ca, PO_4 and OH ions in a special type of lattice configuration. Adherent to the surfaces of the hydroxyapatite crystallites are varying quantities of additional ions including more Ca^{++}, $PO_4^{\equiv}$ plus $CO_3^{=}$, Cl^{-}, Na^{+}, K^{+}, Mg^{++}, citrate, some water (the hydration shell) and traces of other ions. The crystallites are regularly oriented with respect to the collagen in the matrix. This orientation produces much of the form birefringence present in undelcalcified bone sections. All of the above mineral is removed during decalcification of bone specimens and so is not observed by the pathologist on routine preparations.

The second bone fraction is the organic matrix. This is formed by osteoblasts and is composed of about 95% collagen, 5% mucopolysaccharide and traces of other proteins and organic substances. Collagen is orginally formed by the osteoblasts as protocollagen molecules of ultramicroscopic dimensions. After being excreted by the osteoblasts the protocollagen molecules polymerize and aggregate to form fiber bundles of sufficient size to be seen with the light microscope when properly stained. Obscure factors exist which affect the manner in which collagen condenses in the matrix. The presence or absence of these factors is responsible for the difference in formation leading to lamellar or fibrous bone.

The third bone fraction is composed of the cells, vessels and associated organic material normally found in the spaces present in bone. The spaces are the lacunae,

*Aided by Grant No. 293, Henry Ford Hospital Research Funds.

(a) Fibrous bone is variously referred to in the literature as woven, fetal, reactive fiber, immature and primitive bone. These terms all refer to the same substance which is termed fibrous bone in this paper.

canaliculae, Haversian and Volkmann's canals and the medullary space. While this material is the usual object of the pathologist's interest, it is not germane to the present topic and will be ignored henceforth.

The fourth bone fraction is the water normally present in the matrix and bone spaces. This too will be ignored in the following presentation.

We are now ready to consider fibrous and lamellar bone in detail and introduce the topic with:

PHILOSOPHICAL CONSIDERATIONS

Assume that a dump truck deposits a mess of brick, sand and mortar in somebody's yard. There is no overall architectural organization in this mess and this corresponds to the microscopic structure of fibrous bone. Assume that a mason is now hired who constructs a brick wall of the above materials. The finished wall has the same type, quantities and proportions of materials in it as the orginal mess but is now in a highly oriented architectural state. This corresponds to the microscopic structure of lamellar bone.

In summation then, fibrous bone lacks microscopic orientation while lamellar bone possesses microscopic orientation. The analogy falls down in two respects. First, it suggests that lamellar bone is somehow transmuted from fibrous bone when this is not the case. Both types of bone are formed *de novo* by osteoblasts and, once formed, are immutable. One type may replace the other only when the original type has been removed by osteoclasts; the other type is then deposited in the resulting bony defect. Second, the analogy suggests that the identical quantities, types and proportions of "building blocks" in fibrous and lamellar bone is experimentally verified. This is not the case. A great deal is known about the constituents of lamellar bone but little direct examination of fibrous bone has been done and its composition is *assumed* to be similar to lamellar bone.

As far as has been determined from morphology, histochemistry and routine staining behavior no difference between osteoblasts depositing lamellar bone and osteoblasts depositing fibrous bone has been observed.[1,2,3,4,5,8] Nevertheless there must be a difference in their chemical processes. Three cogent bits of evidence support this statement.

First, the structures of the end products are manifestly and consistently different.

Second, one may see fibrous bone being elaborated at one site in a section and lamellar bone being elaborated at another site by a different masses of osteoblasts in the same section but one never observes the same mass of osteoblasts forming both types of bone.

Third, the evolution of the skeleton depicts a clear demarcation between the two bone types. The first skeletons were hyaline cartilage. The second skeletons, representing the next evolutionary step, were of calcified cartilage. The third step in skeletal evolution was the elaboration of fibrous bone and its use to replace calcified cartilage. The final development was the elaboration of lamellar bone and its use to replace

fibrous bone. Each of these steps of course represented a change in the chemical processes of the cells involved.

It is interesting and diagnostically useful that the above sequence of events still obtains in normal and pathological human material. When cartilage is to be replaced by bone it is first calcified, removed by phagocytic cells called chondroclasts and replaced by fibrous bone. Lamellar bone never replaces calcified cartilage directly. Fibrous bone in turn is replaced by lamellar bone under normal circumstances and in this simple fact lies the diagnostic usefulness of the distinction between fibrous and lamellar bone.

CHARACTERISTICS OF FIBROUS AND LAMELLAR BONE: MICROSCOPIC

(A) *FIBROUS:* The collagen in fibrous bone is present as a feltwork of collagen bundles. There is no consistent orientation of these bundles with respect to each other, to the long axis of the bone or to the loads on the part. As a result of this disorganization the pattern observed between crossed polarizers is that of the warp

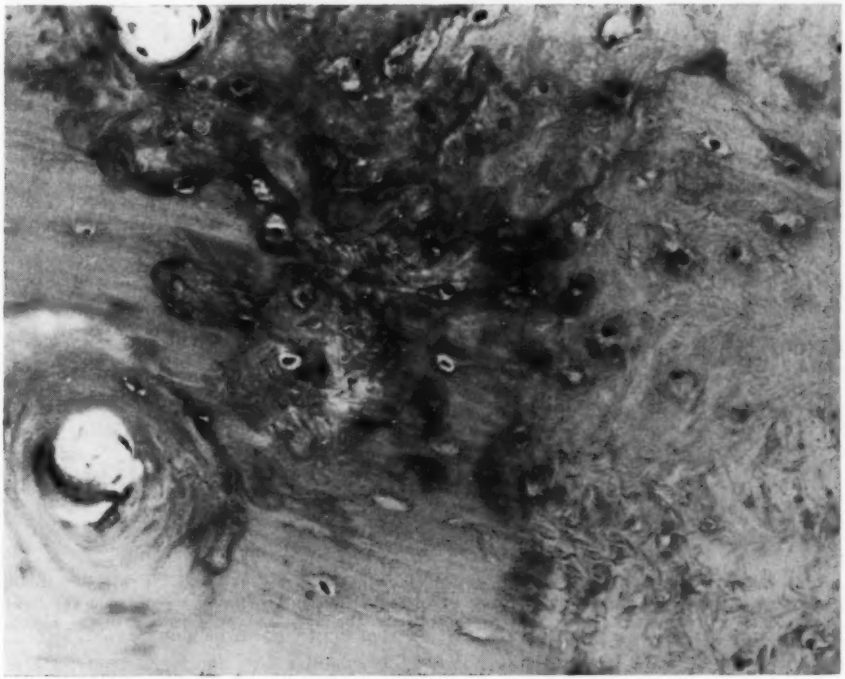

Figure 1

Bright field view of bone from a case of osteomyelitis of low grade severity and several years duration. A diagonal from the upper left corner to the lower right divides the field into two halves. The upper right contains fibrous bone—the black dots are osteocyte nulei lying in the lacunae, which appear as a lighter halo of varying shape and size around the nuclei. The lower left half of the photomicrograph is lamellar bone, with one Haversian system at 8 o'clock. There are fewer lacunae in the lamellar bone and the lacunae are elongated uniformly from left to right.

and woof of cloth, the bright lines being oriented 45° to the "X" or "Y" axes of vibration. A warp and woof pattern in bone examined between crossed polarizers is pathognomonic of fibrous bone.[4]

The lack of orientation of the collagen in fibrous bone is accompanied by lack of orientation of the lacunae in the matrix. Their long axes and flattened sides lie at any random orientation to the optical axis of the microscope. In addition there are considerably more lacunae per unit volume in fibrous bone than in lamellar bone and this difference is readily discernible on sections which contain both types (therefore equal thicknesses which make comparison of the number of visible lacunae per field valid). While there is a similar lack of orientation of the canaliculae, these are seldom apparent in decalcified material prepared in routine ways.[4] (Figs. 1,2,3,4,).

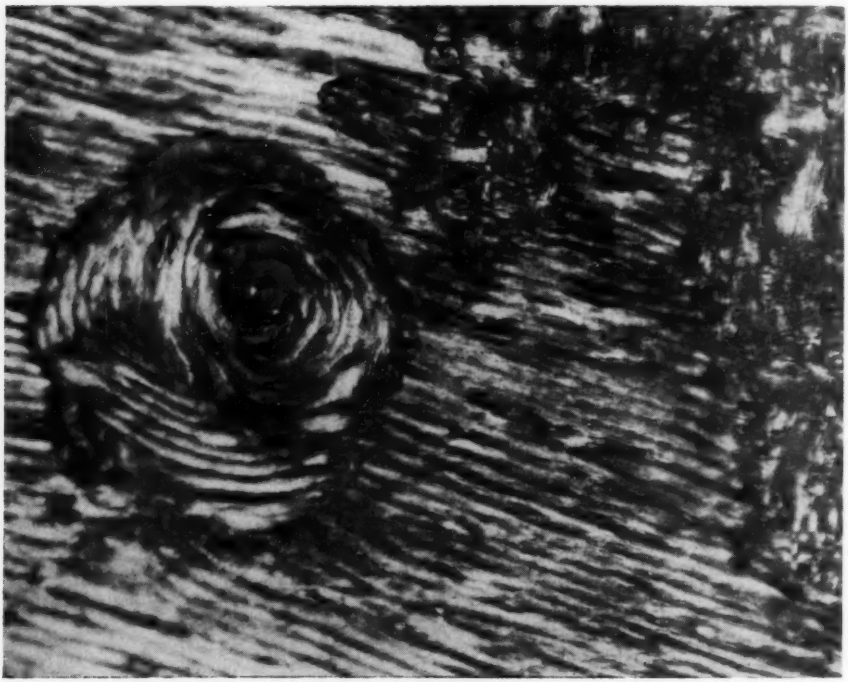

Figure 2

Same field as Figure 1, slightly higher power, crossed polarizers. The lamellar pattern in the bone in the center and to left of center may be appreciated. The warp and woof pattern of the fibrous bone at right margin is not as readily apparent.

Where fibrous bone is being actively formed another characteristic is evident: the irregularity of the surface of the trabeculae of fibrous bone.

The experienced microscopist readily recognizes fibrous bone by the lack of lacunar orientation, the increased numbers of lacunae in fibrous as compared to lamellar bone, the irregular borders of trabeculae, and the lack of any lamellar pattern

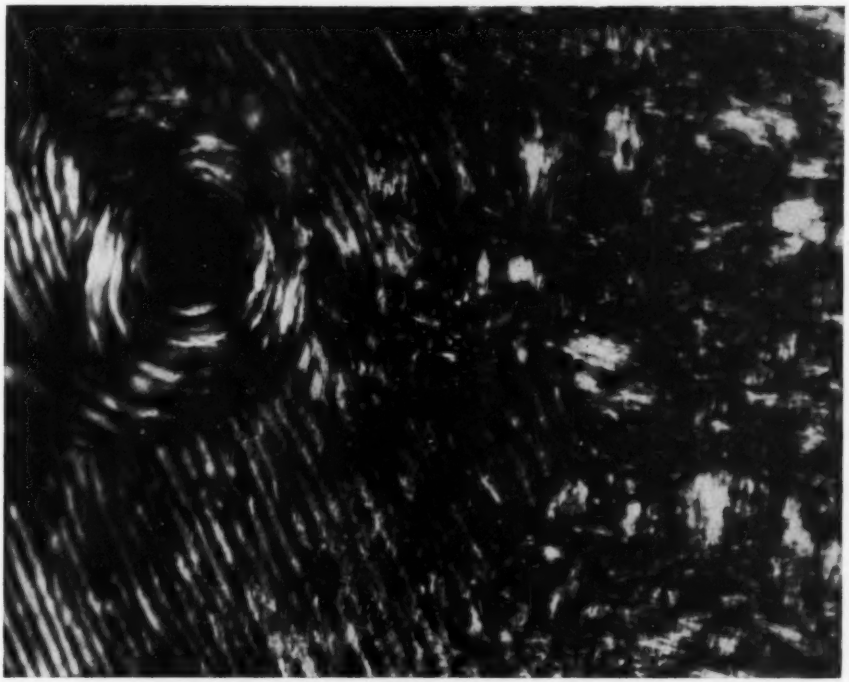

Figure 3

Same field as Figure 2, but rotated clockwise 45°. The warp and woof pattern of the fibrous bone in the right third of the photomicrograph is more clearly evident than in Figure 2.

when fortuitous staining reveals them or when the substage iris is reduced markedly below the numerical aperture of the objective being utilized (this produces a phase contrast effect which is more vivid when proper phase contrast equipment is utilized).

In embryos, fetuses and children the warp and woof pattern is finer than in adults and this has led to the designation fetal bone. Fetal bone is fibrous bone, not a different material.

(B) *LAMELLAR:* The collagen bundles of lammellar bone are finer than those of fibrous bone and are highly oriented with respect to the long axis of the bone, the loads on the part and with respect to adjacent collagen bundles. The result is the typical lamellar appearance between crossed polarizers which is illustrated. (Figs. 2, 3, 5). The lamellae in one bone moiety—for example a single Haversian system—will all have the same orientation with respect to each other. The factors governing this orientation are mysterious, intriguing, and have been the subject of much futile investigation to date. (Figs. 1, 2, 3, 5).

The lacunae in lamellar bone are also oriented, in effect seeming to be squeezed in between lamellae and with their long axes parallel to the long axis of the bone. The usually invisible canaliculae are similarly oriented but run from their lacunae of

Figure 4

Stained, undecalcified cross section human femur. The high degree of canalicular and lacunar orientation in this section, which is almost entirely lamellar bone, may be seen.

At 8 o'clock about ½ way towards the center from the left margin there is a small fragment of fibrous bone whose lacunae and their canaliculae lack the orientation so apparent in the rest of the field. The cause, in this case, of the fibrous bone island was hypertrophic pulmonary osteoarthropathy during the last years of skeletal growth.

origin in perpendicular fashion through lamellae to a vascular channel. There are fewer lacunae per unit volume of matrix than in fibrous bone, the difference being by an approximate factor of 2. The borders of newly forming lamellar bone are smooth and lack the jagged irregularity which characterizes the borders of fibrous bone trabeculae.[4] (Figure 4).

PHYSIOLOGIC

(C) *FIBROUS:* Normally fibrous bone is found only in the epiphyseal plate of children and in the subchondral articular cortex of children and to a lesser degree of adults, and as a spotty layer between the bone cortex and the zone of calcified cartilage at the sites of tendon or fascial attachments to bone. Fibrous bone is the first bone to appear as a bony replacement of cartilaginous anlages in embryos and in the initial formation of membrane bones.[3,5] Fibrous bone which has an exposed surface—i.e.: a surface not covered by lamellar bone or calcified cartilage—is always

replaced by lamellar bone. This process requires considerable time except during the growth process.[4]

Figure 5

Cross section human clavicle, crossed polarizers. The large, markedly oblate Haversian systems with eccentric Haversian canals (black, central oval spots) are typical of young adults. The circular lamellar pattern in the Haversian systems is outlined. The vertical lamellar pattern belongs to the circumferential lamellae. Medullary cavity lies to right.

Fibrous bone is nature's "solder" and in repair, neoplastic, inflammatory and irritative (or reactive) processes is laid down as a rapidly manufacturable material. When the stimulus to production of fibrous bone is mild, little of it is deposited and the trabeculae appear totally unoriented to each other initially. Remodelling processes may eventually produce considerable orientation however. When the stimulus to fibrous bone production is intense the trabeculae begin to show uniform orientation, typical examples being the trabeculae in the sun ray burst of an osteogenic sarcoma, or the onion peel reaction of an Ewing's sarcoma. Again later remodelling processes may considerably alter the initial orientation.

The presence of many haphazard cement lines in a mass of bone is evidence of considerable remodelling activity while lack of these cement lines indicates lack of remodelling. Lack of remodelling is usually due to ischemia or to the relative youth of the bony tissue examined.

Fibrous bone is typically seen in the following situations: normal and abnormal enchondral ossification during growth, elaboration of osteoarthritic osteophytes, ossification in enchondromas, chondrosarcomas and fractures; in infected bone; in repairing bone; as the layer of periostitis or "endosteitis" resulting from a host of irritating processes; and as a peripheral reaction or walling off to various inflammatory or neoplastic processes occurring in or adjacent to bone.

The presence of fibrous bone in other than the normal sites is a positive sign of some pathological process in the skeleton.[4] This sign is dependable even when, for example, biopsy material does not contain the vascular or cytological details and patterns permitting identification of the exact disease.

(D) *LAMELLAR:* This stuff is nature's preferred structural material because it is considerably stronger than fibrous bone. It cannot be elaborated quickly in large quantities. Even a single Haversian system, which is always formed of lamellar bone, requires from 3 to 10 weeks to form.[4] As noted above when fibrous bone exhibits free surface, it is always replaced by lamellar bone under normal circumstances. Thus, for example, the callus which heals a fracture, and which is composed mostly of fibrous bone, is eventually remodelled and replaced by lamellar bone. This process goes on for one or more years after clinical union and restoration of function. In the process small islands of fibrous bone become surrounded and covered on all sides with lamellar bone; in this state they may normally remain for many years. Observation of these surrounded, small islands of fibrous bone is observation of the evidence of some disease or repair process that occurred long ago. (Fig. 4).

The remodelling activity that is a normal part of childhood and adulthood consists of osteoclastic removal of old bone—be it lamellar or fibrous— and the replacement of the old with new bone. Normally this new bone is always lamellar bone. Consequently the osteoblastic activity which interests the endocrinologist, for example, is lamellar osteoblastic activity. The vicissitudes of lamellar osteoblastic activity in various diseases and under the influence of abnormal endocrine factors are not reflected in detectable similar vicissitudes in fibrous osteoblastic activity—another point in evidence that the chemistry of fibrous and lamellar bone formation is different.

SUMMARY

Fibrous bone is unoriented with respect to its collagen bundles and lacunar arrangement and contains more lacunae per unit volume than lamellar bone. Lamellar bone is highly oriented with respect to collagen bundles and lacunar arrangement. Fibrous bone is comparable to solder, quickly elaborated but to be replaced at leisure by lamellar bone which is a better structural material but is slowly elaborated. Normally fibrous bone is seen during growth in restricted areas. The presence of fibrous bone outside of these areas is evidence of a repair or disease process affecting the bone. The pattern of fibrous bone formation, the number of cement lines, and the amount of lamellar bone present yield significant diagnostic information to the pathologist.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases, Philadelphia, Saunders, 1958.

2. Anderson, W. A. D.: Pathology, ed. 2, St. Louis, Mosby, 1953.

3. Arey, L. B.: Developmental Anatomy, ed. 4, Philadelphia, Saunders, 1941.

4. Frost, H. M.: Unpublished work.

5. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 6, Philadelphia, Saunders, 1952.

6. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

7. Snapper, I.: Bone Diseases in Medical Practice, New York, Grune & Stratton, 1957.

8. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.

MEASUREMENT OF OSTEOCYTES PER UNIT VOLUME AND VOLUME COMPONENTS OF OSTEOCYTES AND CANALICULAE IN MAN*

HAROLD M. FROST, M.D.

This work was undertaken to supply measurements of volume components of bone for use in calculations necessitating them.

MATERIALS

These consist of the shafts of long bones from 13 patients, some obtained at autopsy and some at surgery. The bones, ages and sexes are listed in the accompaning chart. Case 13 is from a fibrous dysplastic femur obtained at osteotomy through the courtesy of F. Bost, M.D., deceased, and is not representative of normal bone.

METHODS

Fresh, undecalcified bone sections were made, stained and mounted by the writer's techniques.[3,4]

Volume Components: These were measured by the filar micrometer technique using a Zeiss filar micrometer, Zeiss apochromatic optics of N. A. (numerical aperture) 1.32 fully illuminated with condenser oiled to the slide and Wratten 58 filter in the illuminating beam. Working magnification was 1500 X.

Theory: An infinitely thin plane cut through a well mixed multiphase solid will have percentage area components of each phase in direct proportion to the volume proportions of these phases in the solid, provided the area of the plane is large enough to eliminate random distributional effect. This plane in turn may be sampled by a number of lines drawn at random across it. The total length of the lines crossing each phase will be in proportion to the phase area in the plane and phase volume in the solid, provided enough lines are drawn to reduce random distributional effects to the desired level.[7]

In practice: The extremely thin depth of focus of a large N. A. objective is utilized to produce a sample plane of a bone section.[1,6,9] The depth of focus is some real value on the order of 0.1 u or less. Since canaliculae average 0.4 u in diameter, this depth of focus introduces some error in judging whether a canalicula is just in or just out of focus. Objectives with smaller N. A. and larger depths of focus introduce a hopeless amount of error.[1,6]

Visual contrast is essential for this work and was obtained by the combination of basic fuchsin stain and Wratten 58 filter. The dominant transmission of the filter is 540 mu. Since maximal theoretical resolving power is approximately $\frac{\lambda}{2 \text{ N. A.}}$,[1,9] the theoretical resolving power of the present set-up is about 200 mu. In practice it is better than this, canaliculi only 100 mu in diameter sometimes being resolvable. Resolving power limitations introduce some uncertainty into the measurements.

*Aided by Grant No. 293, Henry Ford Hospital Research Funds.

Osteocyte Per Unit Volume

Filar micrometer technique: The cross hairs of a filar micrometer are utilized to sample the plane image produced by the objective. The cross hairs are run over the section, noting on the drum of the instrument the portions of the run overlying the phase to be measured, and also noting the total length of the run. Separate runs are made by moving the section an arbitrary, fixed amount between runs. A calibrated mechanical stage is useful for this part of the measuring technique. The problem of how many fields and how many micrometer runs must be measured was solved empirically by making an increasing number of runs over the same moiety of bone until the relative variations of separate determinations made up of the same number of runs was less than 10%. 30 runs fulfill this criteria. Random distributional differences in volume components at the necessarily large magnification used often exceed this error by a factor of 20.

In this and the following measurements vascular volume is ignored by the expedient of not making runs where the measurement cuts the location of a vascular channel.

The estimated uncertainty in the canalicular volume component measurements is about 40%, and 10% in the lacunar, most of which is inherent in the nature of light and lenses.

Osteocytes/mm³: Section thickness should be 30-40 u. A calibrated eyepiece micrometer net superimposed on the microscope image provides a divided square similar to that in a heamacytometer. N.A. should be 0.65 or more. Calibration must be done with the objective to be used. All lacunae lying in the net are counted through the thickness of the section, ignoring only those touching the right or bottom borders of the net and those whose lacunae open into the top surface of the section.

Section thickness is measured with the fine motion screw of the microscope by alternately focusing on the upper and lower section surfaces with N.A. of 0.95 or more. An average of 8 determinations is taken, half during the upward and half during the downward traverse of the fine motion. The average is multiplied by the refractive index of undecalcified bone and the mountant $(1.51)^{2,8}$ to obtain the actual thickness of the section.

The number of cells in the measured volume of bone is then converted to cells/mm³ remembering that there are 1×10^9 u³/mm³ and using consistent units.

In measurements done with screw mechanisms the motion of the screw in any one run must always be in the same direction to eliminate backlash, some of which is present in the best instruments.

Shrinkage: The sections measured are dehydrated by warming in air at 35°C prior to mounting. Some shrinkage results. The writer has measured this. The average shrinkage of sections from 6 patients was 2.1% in the tangential and radial and 0.4% in the longitudinal axes of normal bone. It may be as much as 5%, 4% and 1.5% respectively in osteomalacic and rachitic bone. Shrinkage corrections are useless in view of the much larger uncertainty imposed by resolving power limitations.

Error due to inadequate N.A.: The accompanying chart lists some extraneous data obtained through use of objectives of lower N.A. than recommended. The data illustrate the errors resulting from improper fitting of optics to the task required of them. The rather low value for canalicular volume at N.A., .95 in case 2, resulted from an attempt to compensate for the depth of field of the objective. The low counts of cells/mm³ arose from use of low N.A. in measuring thickness, leading to values of thickness about 20% too large.

CASE	AGE	SEX	BONE EXAMINED	NUMERICAL APERTURE	MAGNIFICATION	% VOLUME OSTEOCYTE LACUNAE	% VOLUME CANALICULI	OSTEOCYTE LACUNAE/mm³	NUMBER OF DETERMINATIONS	MAXIMUM RELATIVE VARIATION
1	21	M	Humerus	.32	150			17640	5	16%
1	"	"	"	.65	300			27766	3	5%
1	"	"	Ulna	.32	150			17472	2	4%
1	"	"	"	.65	300			21600	1	—
2	26	M	Tibia	.32	150			17860	1	—
2	"	"	"	.65	300			24500	2	7%
2	"	"	"	.95	1000	3.6	0.66		11	25%
2	"	"	"	1.32	1500	0.94	1.3		28	15%
3	85	M	Tibia	.16	75			16575	2	4%
3	"	"	"	.32	150			18350	1	—
3	"	"	"	.65	300			23162	5	9%
3	"	"	"	1.32	1500	0.73	1.23		28	10%
4	47	F	Tibia	.32	150			16500	1	—
4	"	"	"	.65	300			26200	2	4%
4	"	"	"	.95	1000	5.2	1.2		80	20%
4	"	"	"	1.32	1500	1.10	1.19		28	15%
5	15	F	Tibia	.65	300			25950	2	5%
6	90	F	Tibia	.65	300			27560	5	50%
7	6	F	Clavicle	1.32	1500	0.63	0.79		40	10%
8	38	F	Tibia	1.32	1500	0.56	1.67	(Haversian)	40	30%
8	"	"	"	"	"	0.85	2.7	(Extra-haversian)	40	20%
9	63	M	Tibia	.65	300			25120	3	12%
10	51	M	Tibia	.65	300			26050	2	3%
11	83	M	Tibia	.65	300			30050	2	3%
12	3	M	Tibia	.65	300			29900	2	5%
13	11	M	Femur	.65	300			50220	2	5%
Average Values of Measurements with Maximum Numerical Aperture:										
				1.32	1500	0.80	1.48		204	30%
				.65	300			26100	29	50%

DISCUSSION

The accompanying chart reveals that there is some variation in homogeneity of bone as far as the measured characteristics are concerned. It must be emphasized that the vascular channel volume is excluded from these measurements. If the average values for all the pertinent measurements are to be used in calculations the vascular volume must first be subtracted from the total volume of the cortex, and the lacunar and canalicular volumes then subtracted from the remainder.

The writer has measured vascular volumes in a large number of specimens by the filar micrometer technique. These volumes are usually twice as large near endosteal surfaces as near periosteal, may vary from quadrant to quadrant of the bone, less so along its length, and in special circumstances may exceed 60% of the local cortical volume. Average values for healthy adult cortex range around 6%.

The average lacunar volume component is 0.80% of the cortex. Micropetrotic[5] bone was excluded from the measurements.

The average canalicular volume component is 1.48% of the cortex, micropetrotic bone again excluded.

Lacunae/mm^3 average 26,100.

The average volume of a single lacuna may be calculated and is 3.1×10^{-7} mm^3 or 310^{u3}.

SUMMARY

Using light microscopical techniques it has been determined that osteocyte lacunae average 0.80% and canaliculi 1.48% of vascular-free cortical volume in the long bones of man. There are about 26,100 lacunae/mm^3 and the volume of a single osteocyte lacuna average 310 cubic microns.

REFERENCES

1. Allen, R. M.: The Microscope, New York, D. Van Nostrand, 1940.

2. Ascenzi, A.: Quantitative researches on optical properties of human bone, Nature 163:604, 1949.

3. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

4. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

5. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

6. Hardy, A. C., and Perrin, F. H.: Principles of Optics, New York, McGraw-Hill, 1932.

7. Hennig, A.: Critical survey of volume and surface measurement in microscopy, Zeiss Werkzeitschrift No. 30, Dec. 1958.

8. Lillie, R. D.: Histopathologic Technic and Practical Histochemistry, New York, Blakiston, 1954.

9. Needham, G. H.: Practical Use of the Microscope, Including Photomicroscopy, Springfield, Thomas, 1958.

OBSERVATIONS ON OSTEOID SEAMS*

HAROLD M. FROST, M.D. AND A. R. VILLANUEVA**

Medical literature contains sketchy knowledge of osteoid seams. This paper intends to amplify this knowledge and to correct some misconceptions about osteoid seams. The observations presented were made during the microscopic examination of over 4,500 fresh, undecalcified bone sections prepared by Frost's techniques from more than 300 patients.[3,4] Such sections have significant advantages over routine decalcified H & E sections. The advantages are that the bone is studied as it exists in life; there is less artifact than in sections prepared by any other existing method; osteoid

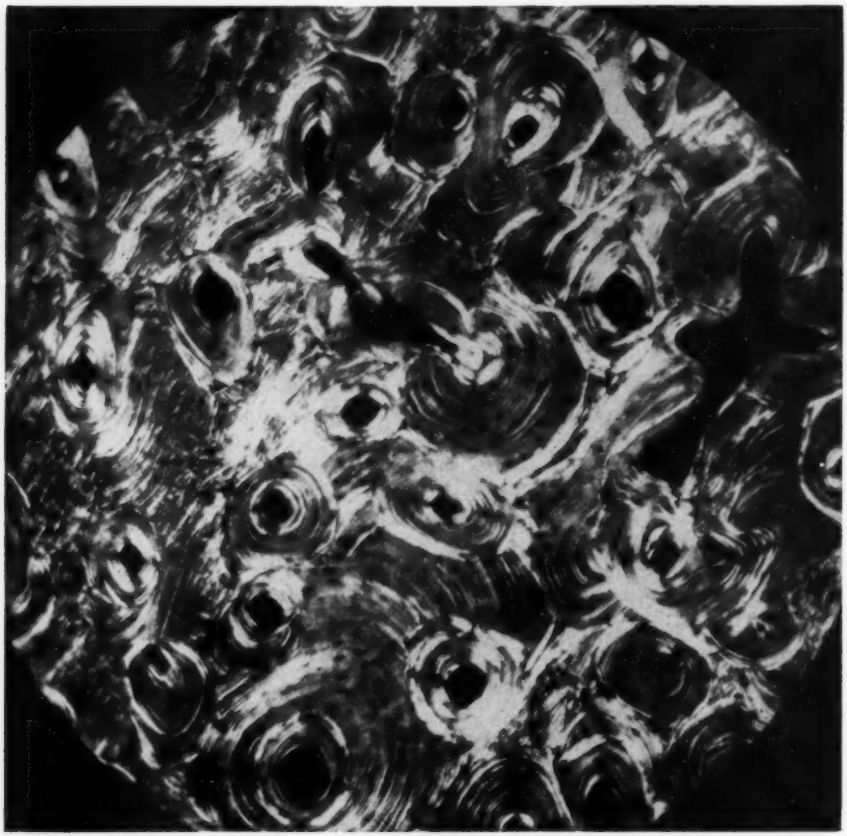

FIGURE 1

Cross section human femur, woman, age 40. About 150 X. Polarized light. The lamellar pattern referred to in section (F) in the text is apparent. The black circles are Haversian canals. These canals are surrounded by the Haversian systems. The lamellae are composed of aggregates of highly oriented collagen bundles impregnated with oriented hydroxyapatite crystals.

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seams are quantitatively demonstrated and quantitatively differentiated from the remainder of the bone (characteristics lacking in orthodox methods for demonstrating osteoid seams[8,11]).[6]

To orient the reader, osteoid seams will be defined.

Bone is formed by cells called osteoblasts. Osteoblasts first form bone matrix which is the organic part of bone. Some time after matrix is formed, it begins to mineralize and once mineralized the mineralized matrix is called bone. During the time interval between formation of the matrix and its mineralization the matrix is

FIGURE 2

Cross section human tibia, about 300 X, polarized light. The dark mass in the center of the circular space at 1:00 o'clock is the shrunken Haversian vessel and surrounding areolar tissue. The dark band lining the wall of the Haversian canal is a similarly shrunken osteoid seam which contains no mineral and is lightly stained with Alizarin. The alternating bright and dark zones beyond the seam are lamellae of mineralized bone.

usually called osteoid or osteoid seam. An osteoid seam is therefore unmineralized organic matrix. There are subtle chemical differences between osteoid and mineralized matrix. For example, osteoid is orthochromatic to toluidine blue stain while mineralized matrix is metachromatic. This and other staining behavior is interpreted to mean a difference exists in the degree of polymerization of the mucopolysaccharide component of osteoid and matrix. Mucopolysaccharides compose about 5% of the matrix.

The major constituent of the organic matrix is collagen—about 95% of the whole. It is felt at present that the osteoblasts form ultramicroscopic protocollagen molecules which are excreted into the cells' environment, and that in response to unknown factors the protocollagen molecules polymerize into crystalline fiber aggregates which may be seen in the light microscope. Fig. 1.

In microscopic sections osteoid seams are seen as layers of unmineralized matrix from 5-30 micra thick, usually applied on a preexisting bone surface but occasionally found free in differentiating tissue. Fig. 2, 3, 4. The use of the term "seam" is semantically unfortunate because osteoid seams are not seams or junctures, but are layers.[1,2,9,10,14]

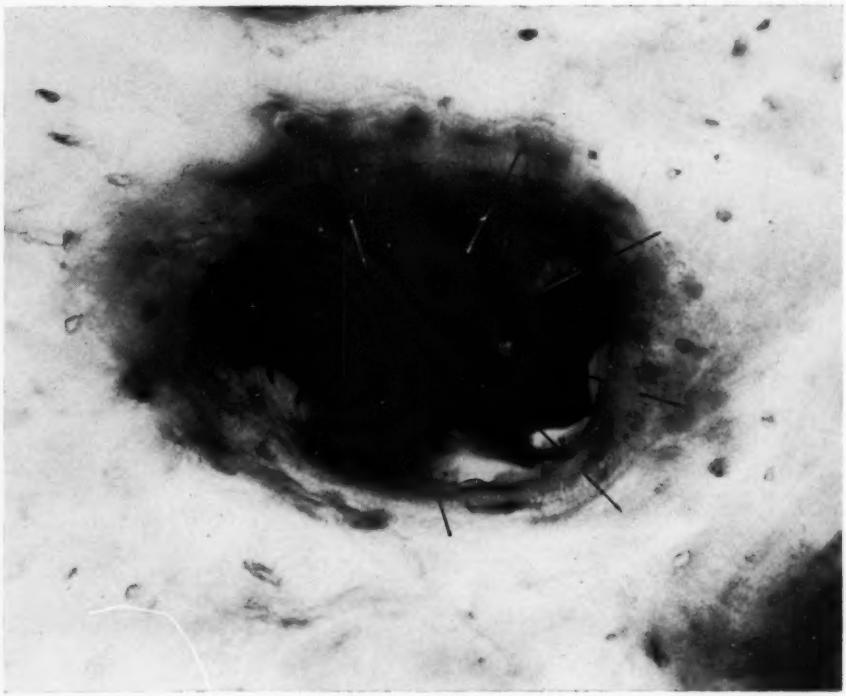

FIGURE 3

Osteoid seam at high magnification. The seam lies between the markers. The black mass in the center of the Haversian canal is grinding debris. Ground section, Frost's Fuchsin.

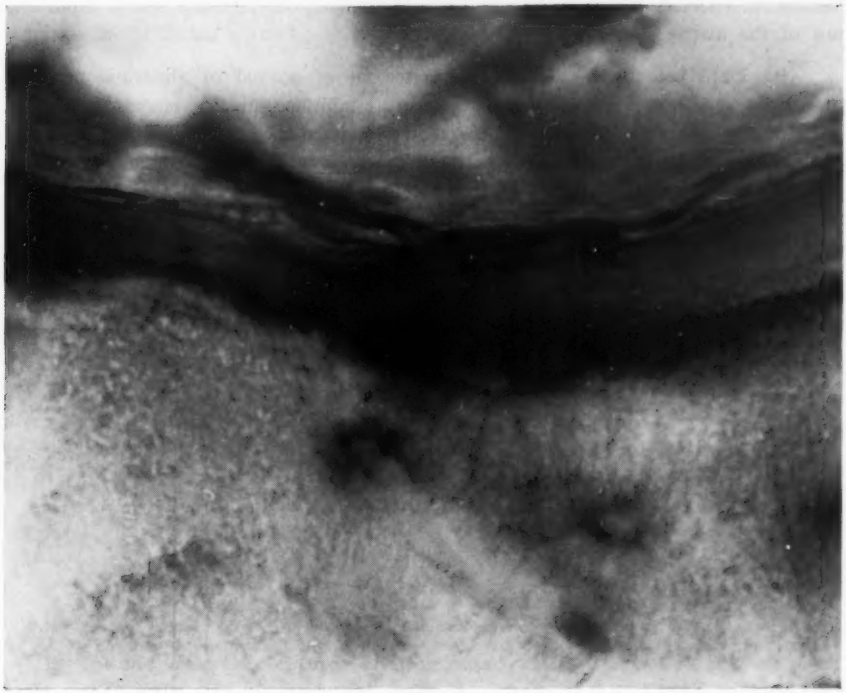

FIGURE 4

High power view of an osteoid seam. The dark border between the seam above and mineralized matrix below is an area where mineral density increases from nearly zero to over 80% of maximal. In the center a saw-tooth border to the mineralized portion is evident. Ground section Frost's Fuchsin.

OBSERVATIONS

GROSS

(A) *Contrary to the tacit, general belief*[1,2,9,10,11,14] *healthy skeletons of all ages and both sexes normally contain osteoid seams.* Absence of osteoid seams at any age and in either sex has in fact been found in this laboratory* to be evidence of a severe disturbance in skeletal physiology. This matter is being dealt with in detail elsewhere.

The normal incidence of osteoid seams—in terms of the percentage of the longitudinal vascular channels in diaphyseal cortex which contain osteoid seams—at age 5 is about 10%; at age 15, 3%; at age 30, 0.8%; and at age 70, 0.8%. There are variations from one bone to another and in general there are higher values in the axial skeleton than in the appendicular skeleton.[6,7]

It can be seen from the above why the presence of seams in adult bone has escaped routine observation. There are normally too few of them—about one per 10 mm² of bone section area—and they are too difficult to discern in routinely stained sections. Special stains for osteoid seams are done as a rule only when metabolic bone

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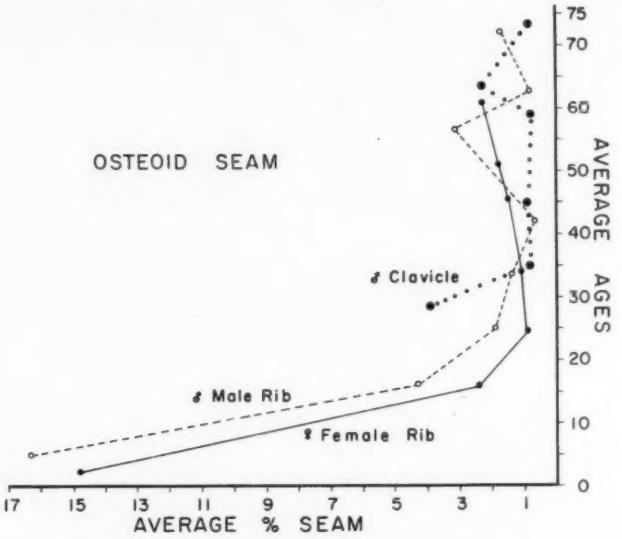
disease is supected. These stains do not quantitatively differentiate seams from the rest of the matrix.[6]

(B) Until recently no quantitative statement of normal or abnormal numbers of osteoid seams in man existed. The norms established in this laboratory change this situation.[7] (See Graph I and II). A variation from the mean by a factor of two to three constitutes skeletal disturbance. For example, a 30 year old man, who would normally have a seam value of 0.15/mm³** in his rib, has a skeletal disturbance if the measured value is 0.40/mm³ or 0.06/mm³. These are changes of such small absolute order that they are impossible to detect without the measuring techniques evolved in this laboratory.[7]



GRAPH I

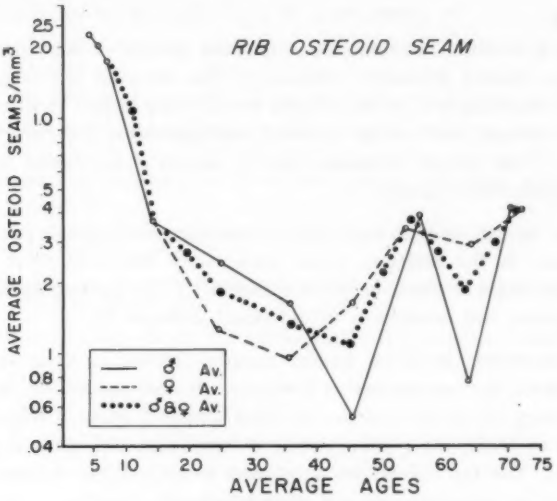
Averages of 75 patients free of cardiac disease and without treatment by cortisone or other hormones in the three months preceding post-mortem, or the operation at which the bone examined was removed. Age is the Y axis and the percentage of the longitudinal channels running in the cortex of the bone which contain an osteoid seam is the X axis. Transversely oriented channels in the cortex are not included. The osteoid seams are demonstrated by methods referred to in the text. It will be noticed that there is rather close correspondence between male and female ribs, the variations probably not being statistically significant in view of the limited number of cases in each ten year age group.

The established norms should greatly potentiate clinical and research diagnostic capability in the field of skeletal physiology.

(C) One thing learned with the aid of the norms referred to is that cortisone and the allied adrenal cortical steroid hormones profoundly depress osteoblastic

**There are cogent reasons considered elsewhere for measuring and expressing osteoblastic activity in terms of unit volume, rather than percent.

activity in the skeleton as manifested by osteoid seams per unit volume of bone. This confirms the predicted effect.



GRAPH II

In this chart the number of osteoid seams per cubic millimeter of bone is plotted against the ages of the 75 patients whose percentage of osteoid seams found in longitudinally coursing vascular channels in cortex was outlined in Graph I. Plotting osteoid seams in terms of per unit volume has proved more desirable than expressing them as per cent of Haversian canals. The reason is that there are variations in channels per unit volume in particular bones and among various ages which introduce unpredictable errors into percentage of seams.

The "W" shaped discontinuity in the right hand portion of the curve is probably significant statistically, but we are unable as yet to determine its cause or its meaning. The Y axis plot is a logarithmic curve, while the X axis is linear. This chart may be regarded as a plot of normal osteoblastic activity in man, by age, with the understanding that osteoid is a product of osteoblastic activity rather than the activity itself.

(D) A second thing learned with the aid of the norms referred to is that osteoblastic activity as manifested by osteoid seams is markedly depressed in severe cardiac failure.

(E) By remodelling activity the skeletal physiologist means the constant destruction of old bone and formation of new bone which is a normal part of mammalian life. Very little hard fact is known about the factors governing this remodelling activity. The ability to quantitatively demonstrate and discern osteoid seams in complete cross sections of any bone enables us to locate and measure this activity. In other words, we may now study remodelling activity with precision. In general, when much new bone forming activity is seen, we may safely infer there is a similar amount of destruction, and location of formative and destructive activities is the same in a broad sense (in absence of local or systemic disease).

In performing seam counts, we have noted that there is a marked preference for seams to occur in the outer half of the cortical thickness, and to concentrate at

the angles of bones such as the tibia and at the sites of attachment of powerful muscles, such as the psoas insertion in the femur. This subject is a field unto itself.

MICROSCOPIC

(F) Normal lamellar bone exhibits a lamellar pattern of birefringence when examined between crossed polarizers. (Figure 1) The observed birefringence is due in part to intrinsic birefringence of the collagen bundles and in part to form birefringence arising in the collagen and in the oriented submicroscopic crystallites. Accordingly the brightness of the pattern decreases when a section is decalcified but it does not disappear or qualitatively change.

About one osteoid seam in four lacks a lamellar birefringence pattern, indicating some disturbance in the collagen, which presumably comprises 95% of the seam. The disturbance might be lack of polymerization of the protocollagen molecules or lack of aggregation and orientation of individual collagen fibers.

(G) Measurements made on human material labelled *in vivo* with tetracycline antibiotics[5] indicate that an average of 0.9 micra of new osteoid per day are formed in actively forming Haversian systems in adults and that about 8 weeks are required for completion of Haversian systems in ill, adult patients. The time might be less for healthy patients. The rate of Haversian system formation has systematic variations, being slowest at the beginning and end of the formative process.

(H) The demarcation between mineralizing matrix and completely unmineralized matrix is not as sharp in undecalcified sections as many histological works indicate. With numerical aperture over 0.75 the advancing front of mineralization resembles a mass of pine cones. The cones consist of mineral accretions about 5 micra long, half as thick at the base with the apex directed towards the Haversian canal. Some of the cones are centered around canaliculae, others around some invisible structure which is probably a collagen bundle.

(I) Decalcified bone matrix stains, albeit poorly, with the silver and metallosulfide methods of Frost while osteoid seams in decalcified or undecalcified sections usually do not. This indicates some difference in the metal binding capacity in seams and mature matrix. Since electronegative dyes (such as Alizarin) exhibit no such preference when used as stains for decalcified sections the suggestion is that the initial mineral binding sites in the matrix are electronegative and that electronegative sites in seams are peculiarly deficient in number.

(J) Well over 10,000 osteoid seams have been observed in the past 7 years. Osteoclastic or other phagocytic destruction of an osteoid seam has not been observed, indicating that if it occurs such destruction is a rare event.

SUMMARY

Some observations on the occurrence and nature of osteoid seams in man have been presented. Of particular note is the normal presence of osteoid seams in human beings of all ages and both sexes, quite contrary to an existing belief that their presence is abnormal in adult life.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases, Philadelphia, Saunders, 1958.

2. Anderson, W. A. D.: Pathology, ed. 2, St. Louis, Mosby, 1953.

3. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

4. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

5. Frost, H. M.: In vivo staining of bone with tetracycline antibiotics, Stain Technol. 35:135, 1960.

6. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. (In press).

7. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in man, Henry Ford Hosp. M. Bull. (In press).

8. Heller-Steinberg, M.: Ground substance, bone salts, and cellular activity in bone formation and destruction, Am. J. Anat. 89:347, 1951.

9. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 6, Philadelphia, Saunders, 1952.

10. McLean, F. C., and Marshall, R. U.: Bone; an Introduction to the Physiology of Skeletal Tissue, Chicago, University of Chicago Press, 1955.

11. Snapper, I.: Bone Diseases in Medical Practice, New York, Grune & Stratton, 1957.

12. Sobel, A. E.: Mechanism of calcification, Bull. Jewish Hosp. 1:5, 1959.

13. Sobel, A. E., and Burger, M.: Calcification. XIV. Investigation of the role of chondroitin sulfate in the calcifying mechanism, Proc. Soc. Exper. Biol. & Med. 87:7, 1954.

14. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.

OBSERVATIONS ON OSTEOID SEAMS: THE EXISTENCE OF A RESTING STATE*

H. M. FROST, M.D.

INTRODUCTION

It has been noted that osteoid seams, contrary to existing belief, are normally present in man at all ages and in both sexes.[7,8] Seams are so infrequent in adults, and current orthodox techniques so unreliable in demonstrating them, that their presence in human adults has largely escaped notice. Very little is known about the qualitative or quantitative aspects of osteoid seam physiology.

It has also been noted that the tetracycline antibiotics become permanently fixed in mineralizing new bone, may be detected in situ in undecalcified sections by several means, and serve as a label on new bone in the same manner as alizarin, radiocalcium and radiophosphorus do.[3,9,10,11,12] The use of tetracycline labelling in man in vivo possesses obvious advantages from the standpoints of safety and convenience. In addition a high proportion of patients coming to postmortem in a large general hospital have had tetracyclines at one or more times in their lives. Sampling their skeletons at postmortem permits one to observe and measure aspects of their osteoblastic physiology characterizing the epoch during which the tetracyclines were given rather than the epoch responsible for their death. As a result, in a large collection of material, study of osteoblastic physiology in health and in numerous diseases becomes possible.

In this paper a resting state of osteoid seams is described. The resting state is a period of time during which progressive mineralization of the seam and the progressive elabration of more matrix is either halted or markedly retarded. Severe cardiac and hepatic failure and prolonged administration of corticosteroid hormones seem to be most often responsible for the causation of the resting state. The resting state was discovered during the examination and measurement of osteoid seams in tetracycline labelled skeletal material from 70 patients. In the majority of resting osteoid seams characteristic changes in staining behavior and morphology occur which permit recognition of the resting seams in properly prepared undecalcified sections without the presence of a tetracycline label.

MATERIALS

The 9 cases utilized in this study are listed in Table I which also notes age, sex and cause of skeletal sampling. Sixteen bones and 43 sections were examined. In all 9 cases tetracycline antibiotics were administered for varying periods up to the day of death or surgery.

METHODS

Fresh, undecalcified sections were made, stained and mounted by the writer's methods.[1,2]

*Work aided by Grant No. 293, Henry Ford Hospital Research Funds.

TABLE 1

CASE NUMBER	105228	135260	135820	529754	747952	807695	854185	866017	955739
Age	57	58	61	12	42	75	66	53	29
Sex	M	M	M	M	M	M	M	F	M
Corticosteroids	0	X	0	0	0	0	X	0	0
Congestive Heart Failure	0	0	X	0	0	X	X	X	0
Tetracycline Dosage, Days, prior to skeletal sample	3	9	5	3	1	6	1	8	3
Bones Examined	Clav Rib	Clav	Rib	Clav Rib	Clav Rib	Clav Rib	Clav Rib	Clav Rib	Fem Tib
Number of Sections Examined	3 3	2	4	2 2	3 2	1 1	3 4	6 3	2 2
Total Seams Observed	10	12	7	6	15	14	5	26	65
Active Seams — Morphology	0	0	0	0	0	9	5	4	20
Active Seams — Tetra	0	0	1	1	0	9	5	0	17
Resting Seams — Morphology	10	12	6	6	15	5	0	22	45
Resting Seams — Tetra	10	12	6	5	15	5	0	26	43
Specimen obtained at Post Mortem	X	X	X	X	X	X	X	X	
Specimen obtained at surgery									X
Number of seams normally expected for this patient and bone(s)	6 3	12	6	12 3	18 3	6 1	18 6	36 5	40 20
Totals	9	12	6	15	21	7	24	41	60
Primary Cause of Death or Surgery	Hepatic Failure	Hepatic Failure	Metastatic Ca	Biliary Cirrhosis Atresia Con. Duct	Hepatic Failure	Leukemia	Pulm. edema	Pulm. edema	Ostamer Tibia

The appearance of osteoid seams in the writer's sections has has been discussed elsewhere.[4,7,8] They are illustrated in Figures 1 and 2 in this paper. The methods recommended quantitatively stain seams and permit their quantitative differentiation. In this work seams were counted by systematic searching of the complete cross sections of the listed bones, using a mechanical stage on the microscope and N.A. in excess of 0.3.

Only complete cross sections were examined due to normal distributional effects which make longitudinal sections less suitable.[5,6,8]

The presence or absence of tetracycline in the sections examined was determined by using the writer's microfluorescence set-up.[3] While the quantitative limits of this method are not known, the method is extremely sensitive.

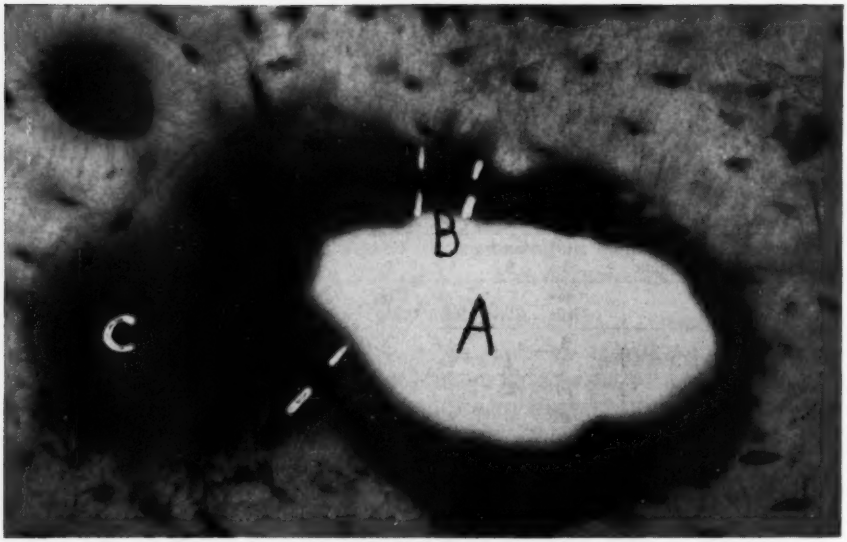


FIGURE 1

Case 807695. Clavicle, cross section, undecal., basic fuchsin, Normal, morphologically active osteoid seam. The Haversian canal is marked at (A). The osteoid seam is marked at (B). The granular, fuchsin permeable bone at the periphery of the seam is marked at (C). A fluorescent, tetracycline band is present (but not illustrated here) at the periphery of the seam.

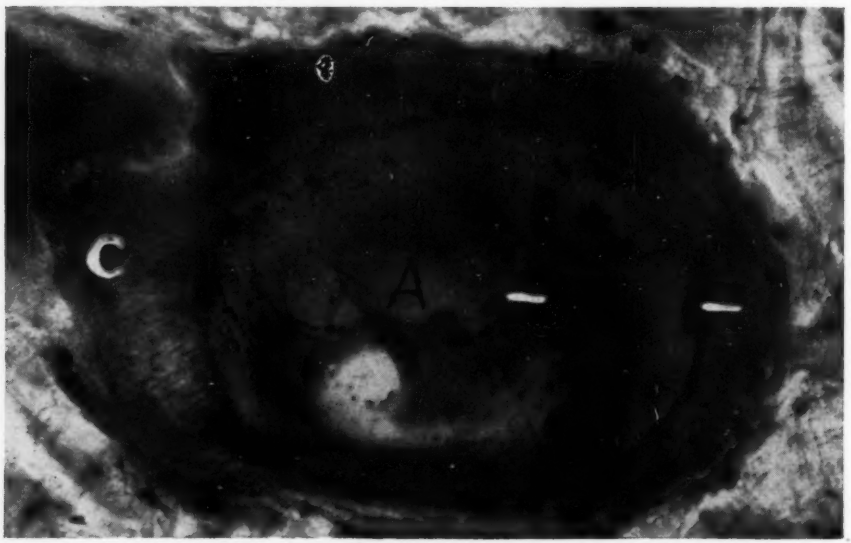


FIGURE 2

Same Section. A morphologically resting seam. Loss of the fuchsin permeability and granularity of the bone at the periphery of the seam is accompanied by failure, over 6 days of administration, to acquire a tetracycline band at the periphery of this seam.

OBSERVATIONS

In Figure 1, a "normal' active osteoid seam is illustrated. This is the usual appearance of actively mineralizing osteoid seams in human material.* The fuchsin staining, granular bone at the periphery of the seam is characteristic of active seams.

In cases 807695, 854185 and 955739 these active seams are identified by a tetracycline band at the periphery of the seam.

In Figure 2, a resting, inactive seam is illustrated. Note that there is a great deal *less fuchsin permeability,* and much *less granularity,* of the bone at the edge of the seam. Of all of the 111 seams which appeared on a morphological basis to be resting in the cases of Table I, only 4 were active as indicated by the presence of a tetracycline band at the periphery of the seam. Of the 38 seams which appeared active by the above morphological criteria only 7 were actually inactive as indicated by the lack of a tetracycline band at the periphery of the seam.

DISCUSSION

It is not clear from the observations listed whether mineralization is completely arrested or just markedly retarded in the specimens containing resting seams. Regardless of which is the case, it is significant that a mineralization abnormality has been detected in other disease processes than rickets or osteomalacia.

It is significant too that elaboration of the osteoid appeared to stop also, so that the seams which were observed were not of excess thickness. The exception is case 807695, in which some thick seams were observed.

As might be anticipated from the biological—as opposed to inorganic-nature of the phenomena being considered, the data, while conclusive enough to establish the existence of a resting state of osteoid seams, exhibit some exceptions which are unexplained and suggest that deeper understanding of the matter is needed. What mechanism or mechanisms are involved in causing the resting state in a seam? Are they local as well as sytemic, are they endocrine, physiochemical or what? How can congestive heart failure result in diminished or arrested mineralization of new matrix? How can it also result in diminished or arrested formation of matrix? These are matters for more work. The prospect presents itself that answers to these questions might illuminate the mechanisms of seemingly unconnected processes, for example, senile osteoporosis (supposedly also a failure of matrix elaboration) or the slow healing of fractures in adults as opposed to children (a retardation in matrix elaboration).

SUMMARY

With the aid of tetracycline labelling in vivo, it has been shown in undecalcified human bone sections that a resting state of osteoid seams occurs. The resting state is morphologically identifiable in most cases and physiologically is characterized by arrest in mineralization of matrix and arrest in formation of matrix.

*Activity indicted by the presence of a tetracycline band in sections from patients receiving the drug.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

2. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

3. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

4. Frost, H. M.: Some observations on bone mineral in a case of vitamin D resistant rickets, Henry Ford Hosp. M. Bull. 6:300, 1958.

5. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

6. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

7. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

8. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. (In press).

9. Frost, H. M., Villanueva, A. R., and Roth, H.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

10. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracyclines, Henry Ford Hosp. M. Bull. 8:255, 1960.

11. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

12. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of the tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

IN VIVO IMPERMEABILITY OF FEATHERED BONE TO TETRACYCLINES*

HAROLD M. FROST, M.D.

INTRODUCTION

The existence of an incompletely mineralized state of some bone matrix in man *in vivo* has been separately reported. This incompletely mineralized state has been termed *feathering*[3] and is not causally or essentially related to osteoporotic states or osteomalacic states of bone. Serum chemical values of calcium, phosphorus, alkaline and acid phosphatase are usually normal in patients with severe degrees of feathering.

It has been reported that feathered bone in vitro is highly permeable to a large number of inorganic and organic reagents tested in this respect.[3,5,8] Milch, Rall and Tobie deserve the basic credit for noting and publishing the fixation of tetracyclines in bone.[10,11]

The use of tetracyclines *in vivo* in man to label bone and explore some aspects of permeability and diffusion impedence in bone has been reported.[4,8] This paper describes the *in vivo* impermeability of feathered bone to tetracyclines, a result diametrically opposite of that expected.

MATERIAL

In the Henry Ford Hospital Orthopaedic Research Laboratory case material, sections were reviewed from 43 patients who received tetracyclines during life. Of these 43 cases, drug dosage had ceased in 37 cases two or more days before death or the surgery from which the specimen was obtained. In 6 cases tetracycline administration continued up to the time of death.

The cases in which drug dosage stopped some time before death or surgery supply material from which we can learn where and in what quantity tetracyclines are permanently fixed in bone.[4,9]

The cases in which drug dosage continued up to the time of death or surgery supply material from which we can learn where and in what quantity tetracyclines reversibly stain tissue but from which they disappear upon cessation of drug dosage.[4,9]

METHODS

For the present purpose a microfluorescence set-up was used to detect the presence of tetracyclines. The microfluorescence method is much more sensitive than detection of tetracycline with bright field technique. Consult refs. (4, 6) for fuller details.

With microfluorescence methods tetracyclines appear white, yellow or yellow-orange on a green to magenta background. They are readily recognized in suitably prepared material by their color. Only a few cases have been found where other substances administered to patients *in vivo* cause fluorescence in suitably prepared material.

From the bone specimens submitted for examination thin, undecalcified sections

*Aided by Grant 293, Henry Ford Hospital Research Funds.

were made, stained with basic fuchsin and mounted according to the writer's techniques, which should be closely adhered to for this type of work.[1,2]

OBSERVATIONS

A. *Cases where tetracycline dosage continued to time of death or surgery:*

Detection of tetracycline in these patients' tissues indicates two things: A diffusion pathway was open to the tetracycline molecules in the blood, and some mechanism existed for attachment of considerable numbers of these molecules to the material where fluorescence is observed.

New Bone[a] Tetracycline is detectable in over half of the fuchsin-permeable portions of new bone. There is a distinct perilacunar preference for the maximum observable concentration of the drug.

Feathered bone[b] On the average only about 5% of the feathered osteons in these cases exhibited any detectable fluorescence. Since the new and feathered osteons were observed in the same sections, vagaries of technique cannot account for the difference which must be real rather than artifact. There is, of course, a limit to the amount of tetracycline which can be detected in bone by microfluorescence. We do not know quantitatively what this limit is, but it is on the order of millimicrograms when proper microscopic technique is excercised.

B. *Cases where tetracycline dosage stopped some time before death or surgery:*

New Bone: No tetracycline can be detected by the means utilized in any new bone in these 37 cases.

Feathered bone: A small percentage of feathered osteons contained detectable tetracycline. The percentage varied, but is on the order of 0-7%. The majority of feathered bone revealed no tetracycline. Interestingly, sizable numbers of osteons were observed in which the feathered portion revealed no tetracycline while the normally mineralized portion revealed a tetracycline band, indicating the osteon was in the formative process during drug dosage. This enables us to date such osteons if we know when the drug was given. In the two oldest cases, tetracyclines had been administered 6 years before death and the bands are still present, though faded somewhat. Several of these 6 year-old osteons are also feathered, proving conclusively that feathered bone is not necessarily new bone.

DISCUSSION

The foregoing observations indicate that the vast majority of feathered bone in man is not permeable to tetracyclines in vivo. With due caution in interpretation,[c] this phenomenon suggests that some diffusion barrier exists in feathered bone which might

(a) Considered to be new by criteria of the presence of osteoid seams lining the vascular canal or by empty lacunar counts of 2% or less. See references (3, 7) for details.

(b) Considered to be feathered according to the criteria in reference (3).

(c) The multiplicity of factors which may affect bone permeability and blood-bone exchange are discussed elsewhere but make an assumption of equivalent behavior between tetracycline and other molecules rather dubious without any corroboratory evidence.

also affect the permeability of feathered bone to other substances such as lead, strontium, calcium and the normal electrolytes and buffers found in the blood.

Such a diffusion barrier might also help to explain how feathered bone can exist in the skeleton for such long time periods. Current thinking indicates that, once mineralization of the matrix is initiated, it should continue progressively even in the absence of living bone cells, as long as normal body fluids bathe the bone. If this were the case, how could old feathered bone exist? A diffusion barrier between the feathered bone and the vascular fluids would explain the situation, at least in part.

Nothing outlined in this paper suggests itself as an explanation for the diffusion barrier hypothecated.

SUMMARY

Using *in vivo* staining of bone with tetracyclines, it has been found that feathered bone *in vivo* is, for the most part, impermeable to tetracyclines. This suggests that *in vivo* some diffusion barrier exists which isolates feathered bone from the blood. It has also been learned that at least some feathered bone is 6 years old.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

2. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

3. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

4. Frost, H. M.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

5. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

6. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

7. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

8. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:263, 1960.

9. Frost, H. M., Villanueva, A. R., and Roth, H.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

10. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

11. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of the tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

HALO VOLUME*

HAROLD M. FROST, M.D., A. R. VILLANUEVA AND H. ROTH**

This paper presents evidence for the existence and dependence on osteocyte metabolism of a feature existing in perfectly fresh undecalcified bone sections and first described by Frost in 1957.[1] The feature is termed halo volume because it exists in three dimensional space as a "halo" around living osteocytes and adjacent parts of their canaliculae. H. V.'s (halo volume's) most important feature is a quasipermeable state of the bone wall of lacunae and canaliculae. It is normal characteristic of fresh bone.

The majority of the evidence presented was elucidated by us but additional and prior evidence from other laboratories exists and will be presented briefly.

MATERIALS

Bones from over 100 humans of all age decades and both sexes plus bones from over 30 dogs, sheep, monkeys, rats, rabbits and mice have been examined by the techniques which will be mentioned. In the majority of the cases the cortex of long bones was examined but cancellous bone from metaphyses and axial skeleton have also been examined.

METHODS

Undecalcified, fresh bone sections are made and stained for H. V. by Frost's techniques.[3,4] These techniques have the important requisite of permitting examination of bone with introduction of less artifact than other existing methods. Several peculiar aspects of H. V. demonstration will be briefly mentioned.

Bones must be examined no more than 6 hours after death or removal at surgery. During this time drying must be prevented by immersing bulk material in tap water, distilled water, normal saline or Ringer's solution.

The following treatments consistently cause the majority of H. V. to disappear and the remainder to be smaller than normal in experimental sections as compared to control sections kept in distilled water at room temperature: Immersion in methanol, ethanol, propanol, isopropanol, acetone, dioxane, glycerine, formalin, heavy metal fixatives, solutions with unphysiologic pH's or molarity, solutions containing ions which form insoluble precipitates with bone salt ions; freezing; refrigeration above freezing; drying for over 30 seconds; storage longer than 6 hours. It is astonishing that fresh material should contain a feature so sensitive to such a number of procedures in common histopathological use. We believe this explains why H. V. have escaped prior notice.

Frost's paper details several methods for staining H. V.[4] The permanganate method is the simplest, quickest and has been completely reliable in our hands. In brief, a freshly made section is boiled 12 minutes in 0.1 N $KMnO_4$. Grind off all resulting

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stain from the bottom and part from the top of the section. Dry on a warming plate and mount in neutral synthetic resin. The mount is permanent. Section thickness is immaterial. Only the surface 10-20 u are stained, the remainder of the section serving as a mechanical carrier for the stained portion.

The following orientation is given for the reader unfamiliar with stained, undecalcified sections.

Since no definition of normal or abnormal degree of mineralization exists, we have adopted Frost's arbitrary standard of permeability to 40% alcoholic basic fuchsin. "Normally' mineralized bone is not permeable, "incompletely" mineralized bone is permeable to this reagent. Numerous other histological dyes tested behave in the same manner as basic fuchsin.[4]

Normally mineralized bone stained with these reagents reveals unstained interlacunar and intercanalicular bone, stain on the walls of the physiologic spaces in bone and stain of the protoplasmic contents of these spaces. Failure to grind off surface stain prior to mounting has led to the erroneous and widespread impression that diffuse permeability to the organic histological dyes exists in undecalcified sections.

Within the inherent optical limitations (0.95 N. A., $\pm$ 0.3 u resolution) the size of lacunae and canaliculae in fuchsin stained and in air mounted sections is the same.

HALO VOLUME DESCRIPTION

H. V. is the 0.5 — 2.0 u depth of bone wall of the lacunar and canalicular lumens. This part of the bone wall normally is permeable to small but not to large ions and is stained by the recommended methods. This selective permeability is the major observable feature of H. V. Because the recommended permanganate stain penetrates into the bone wall of the lumens, the lacunae and canaliculae appear larger in H. V. stained sections than in fuchsin stained sections from the same case. (See Fig. 1, 2).

With the permanganate method outlined, H. V. have been demonstrated in all of the material listed under Materials. One's immediate reaction is that this is artifact resulting from the elevated temperature and from the fact that the lumens are the easiest access routes for the stain reagent.

The suspicion of artifact is dealt with by the 11 observations next presented.

1. H. V. is demonstrated by other techniques using room temperature, different reagents and with anionic and cationic reagents. Some substance(s) may be selectively leached out of the H. V. moiety of bone without affecting the remainder. Blocking phenomena in H. V. can be produced.[4]
2. No H. V. stain pattern results with the large moleculed dyes tested with the single exception of sodium alizarin sulfonate. This is true at room temperature and at 37° C.
3. Control sections of fresh, live bone invariably reveal H. V. with the per-

manganate technique recommended while experimental sections variously treated as listed under Methods lose the majority of the H. V., the remainder being smaller. The only exception has been bone from a case of vitamin D resistant rickets whose very large H. V. were not noticeably affected by alcohol or storage time.

The above behavior suggests some physical change in the H. V. bone moiety as a result of the described treatments. As a result of this change, small ion permeability is drastically reduced in H. V.

4. Micropetrosis and osteocyte death in vivo have been discussed in other papers by Frost,[6,7] by Jaffe and Pomerans[10] and by Sherman and Shelakovitch.[14]

   (a) Micropetrotic bone is dead. H. V. cannot be stained in micropetrotic bone.

   (b) There are usually fewer live osteocytes in extraHaversian bone than in Haversian. There are usually fewer H. V. stained in extraHaversian bone than in Haversian.

   These facts suggest that a live osteocyte is a requisite for H. V.

5. No. H. V. can be stained with the recommended techniques in sections decalcified, prior to staining, in dilute nitric, hydrochloric or acetic acids or by citrate complexing. No H. V. stain is seen in routine H&E bone preparations.

6. The parts of canaliculae up to about 20 u from the osteocyte show H. V. stain but the parts farther away normally do not, in spite of the fact that generically all parts of canaliculae are exposed to the section surface at one or another point. The silver and lead sulfide methods are occasional exceptions to this rule and sodium alizarin sulfonate a frequent one.

   Again some physical difference between H. V. staining and non-H. V. staining bone must exist to explain this difference in behavior.

7. Fig. 1 is a photomicrograph of a fuchsin stained cross section from a 72 year old man dying of gastric hemorrhage. Fig. 2 is of a permanganate stained section of the same bone at the same magnification. Fig. 3 is another permanganate stain done after storing the bone 48 hours in 40% ethanol. The apparently larger size of the lacunae and canaliculae in Fig. 2 as compared to Fig. 1 is evident. The loss of H. V. in Fig. 3 following storage is equally evident. Less than one minute in alcohol is needed to initiate this difference.

8. Fig. 4 illustrates the H. V. in a control rat immediately after sacrifice. Fig. 5 illustrates the H. V. in a littermate given 1000 units of parathormone parenterally 12 hours before sacrifice. The H. V. are more numerous, larger and affect a greater length of the canaliculae. Some areas in Fig. 5 are micropetrotic and are free of H. V.

9. Fig. 6 illustrates a CoS stain on a section from a boy with vitamin D resistant rickets. The specimen was obtained at osteotomy through the courtesy of

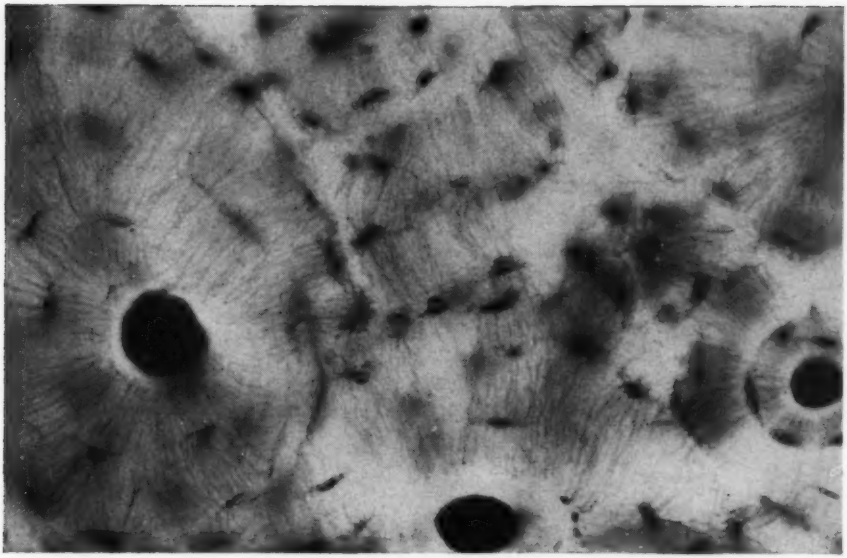

Figure 1

Cross section clavicle 72 yr. male. 0.95 N. A., 400X. Stained with basic fuchsin. The dark band around the osteocyte lacunae is fuchsin and represents the true size of the lacunae. The canaliculae are barely resolved.

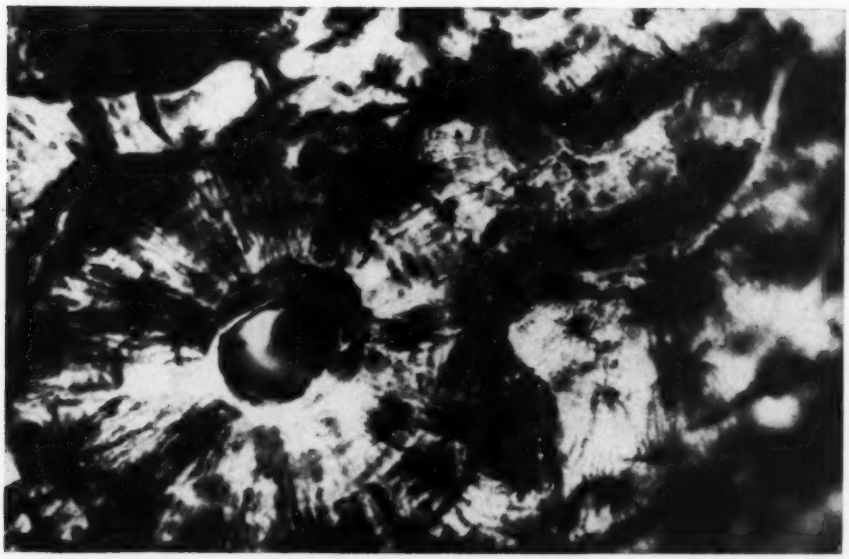

Figure 2

Cross section same bone, same optical factors. Stained with permanganate as recommended in the text. The "spiders" in the Haversian system to the left and below the center are halo volumes. The permanganate has penetrated into the bone wall around the lacunar and canalicular lumens giving the impression they are a great deal larger than they actaully are. Compare with Fig. 1.

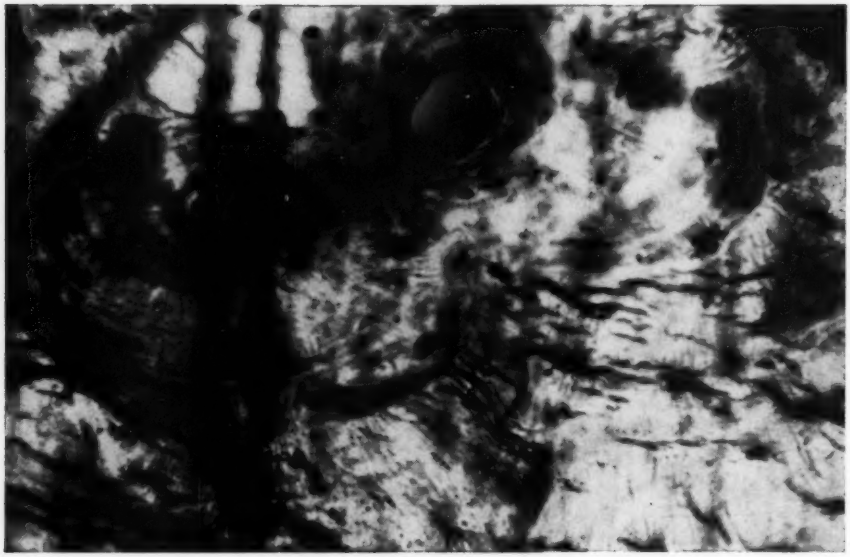

Figure 3

Cross section same bone, permanganate stain but done two days after death of patient. The storage time has caused the permeability in the H. V. which was present in the perfectly fresh material (see Fig. 2) to disappear for the most part. The vertical markings to the left of center are surface stain left on to prove that the difference is not due to grinding off more material in this section than in that of Fig. 2, which actually had more surface removed than the present section.

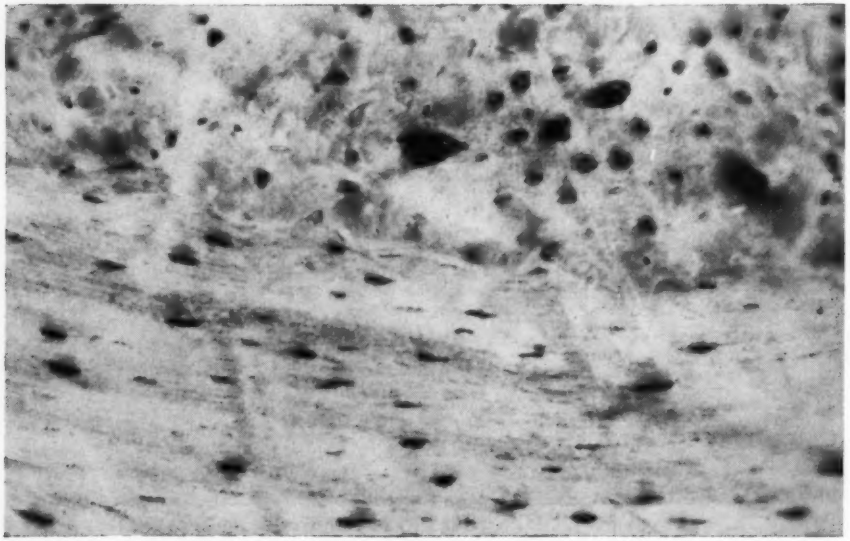

Figure 4

Cross section M/3 femur control rat, permanganate stain. Same optical factors as in Figs. 1-3. Very poor stain for H. V. Vertical striations are identification of surface grinding marks as in Fig. 4.

Figure 5

Same stain and optical factors but rat given 1000 units parathormone 12 hrs. before death. Large H. V. around lacunae and canaliculae. The clearer bone below and to left of center is micropetrotic.

J. D. Fleming, M.D. The large, black blurs represent markedly enlarged H. V. Fig. 7 illustrates a fuchsin stained section from the same case. The dark blurs represent fuchsin permeability around osteocytes. Fuchsin permeable H. V. are rare in the experience of this laboratory. Further details of this case and the findings are published.[5]

10. Examination of fresh, unstained sections in water mounts with the Baker A-0 interference microscope reveals that there is a shorter optical path in the H. V. bone compared to the surround. The volume of bone so affected is usually larger by a factor of about 3 than that stained with the permanganate method. After drying and mounting in synthetic resin most of the H. V. detectable by interferometry disappear, the remainder becoming smaller than they previously were.

    Dilute, water soluble stains such as methylene blue may be used to permit identification of lacunae which are empty of protoplasm in water mounted sections. Since the time required for staining beyond the surface 10 u of the sections is too long for preservation of H. V., this method is confined automatically to examination of lacunae close to the surface of the section. Empty lacunae do not usually manifest H. V. and full ones usually do. There are a certain number of "sports, varying from 0.5-3.0% which violate the above rule.

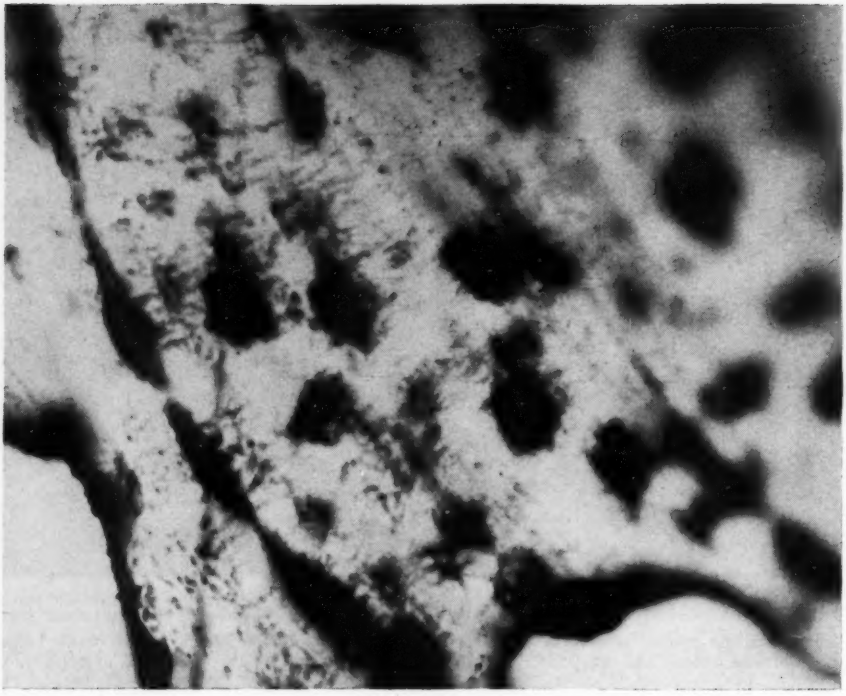

Figure 6 .

Colbalt Sulfide stain tibia 9 yr. male with vitamin D resistant rickets. 0.95 N. A. 600 X. The large black blurs are enlarged H. V. A few much smaller H. V. are interspersed which give some idea of the true lacunar dimensions.

Interferometry is an important confirmatory technique for the thesis of this paper since the image produced in the interference microscope depends on an inherent difference in refractive index among the various components of the object. The staining techniques depend on permeability and if this were our sole evidence, suspicion of artifact would be justifiably entertained.

11. Initially we mentioned that other authors have published evidence for existence of H. V.

    (a) Heller-Steinberg published fascinating work in 1951 done on fresh, undecalcified frozen-dried bone.[9] With a silver technique, PAS and toluidine blue she was able to produce, describe and illustrate enlarged H. V. in a group of rats given parathormone as compared to a control group. She speculated that the parathormone might have exerted a regulatory effect on the osteocyte metabolism. Silberberg and Silberberg later duplicated successfully parts of Heller-Steinberg's work.[15]

    (b) Several authors have published high resolution microradiographs of bone from cases of vitamin D resistant rickets. The current example is

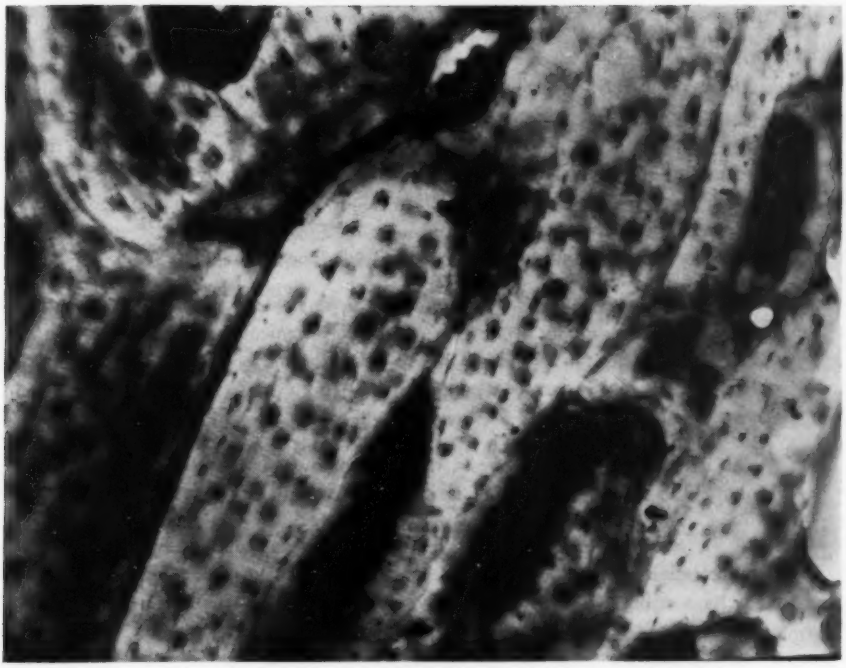

Figure 7

Fuchsin stained section from same case as in Fig. 6. 0.65 N. A. 200X. The very dark, broad vertical striae are volumes of fuchsin permeable bone. The small dark blurs scattered over the field are enlarged H. V. which are fuchsin permeable. On the original some canaliculae are resolved and the osteocyte nuclei can be seen as tiny, very dark dots in the center of lacunae. Where fuchsin permeates into the bone mineralization is defective in amount per unit volume. As a result microradiographs of vitamin D resistant rachitic bone also reveal a dark blur in the H. V. due in this case to lack of obstruction of the x-ray beam.

the paper by Engfeldt and coworkers.[2] In their illustrations the radiolucent H. V. around many of the osteocytes present is manifested as a dark blur very similar to Fig 7 in this paper. Their imagery depends on physical absorption by calcium nuclei of gamma rays from a monochromatic source while ours depends on permeability to an organic dye. The convergence of findings with such different methods is striking to us.

DISCUSSION

The foregoing evidence, derived from three completely different physical methodologies in three independent laboratories, indicates that there is something different about the 0.5 - 2.0 u of bone comprising the wall of the osteocyte and canalicular lumens which contain living cells. By and large this differences disappears with the death of the cell and the necessary inference is that the metabolic activity of the cell is somehow responsible for it. Examples of pathological distortion of this bone wall, the halo volume, are given, suggesting that further study of this cell would be fruitful.

Five specific points merit comment.

1. *Unstable Physical State:* The surprising sensitivity of the permeability and difference in refractive index characteristic of H. V. in perfectly fresh material to procedures in common use brings Dallemagne's work and views to mind.[1] He feels that the in vivo ultrastructure of the bone mineral is not hydroxyapatite but some other state. Conversion to hydroxyapatite occurs after death since this is the stable structure in the absence of the (unknown) living chemical environment. Accordingly he feels that most of the investigations done on bone salt ultrastructure have been done on artifact.

   Our present work has demonstrated some unstable state in H. V., which is permeable in perfectly fresh material but impermeable after numerous treatments which are in common use in tissue laboratories. It must remain for future work to reveal if Dallemagne's structural concepts are valid. It is possible that both he and his critics will prove partially correct.

   The H. V. sensitivity suggests that it would be worthwhile to check such matters as x-ray diffraction patterns on fresh, still moist bone.

2. *H. V. and Parathormone:* In view of the enlarged H. V. in rats after administration of parathormone it seems reasonable to assume that parathormone exerts a definite effect on osteocyte metabolism which in turn causes enlargement of H. V. One further step leads to the suspicion that some of the skeletal calcium load cast out through the urine in hyperparathyroidism arises in H. V. rather than through osteoclastic resorption of bone. Citrate metabolism has often been suggested as the chemical route by means of which this loss might occur.[11,12,13]

3. *Size Effect:* The consistent discrepancy in H. V. permeability between large and small ions suggests a size discriminatory factor operates in normally mineralized bone. The effect is that of reduction in permeability to progressively smaller ions as mineral density progressively approaches its maximum. The causes of this effect other than mineral density and ionic size are not apparent and are probably complex. This effect has been suspected previously[11] and we have now provided direct evidence for its existence. A size discriminatory effect would have important applications to blood-bone exchange processes and the physiology of osteocyte, osteoblast and osteoclast.

4. *H. V. and Blood-Bone Exchange:* The observed failure of the major fraction of a bone ion-sodium for example—to equilibrate with an injected radioactive tracer was a major factor in the development of the defect crystal theory which holds that the nonequilibrating fraction is locked in the crystal lattice or hydration shell.[12] Inherent in this theory is the assumption that the tracer has free access to the crystal if not to its individual atoms.

   More than a dozen anions and cations tested in this laboratory failed to permeate beyond the H. V. in 48 hours.[4] This suggests that the assumed ready

access of all the crystallites to an injected tracer assumes too much, a suspicion others have also entertained.[11]

We suggest that there are other factors which affect blood-bone exchange in addition to the purely passive diffusion of ions among the crystallites and that H. V. is one of them. In effect, H. V. would be a quickly available reservoir of bone permeable to small ions, adjacent to the osteocyte and the diffusion gradients which enable it to live, and thus a more ready donor or acceptor volume of bone in time of need than the remainder of the intercanalicular and interlacunar bone, which in vitro at least is highly impermeable over 48 hour periods.

5. *H. V. and Vitamin D Resistant Rickets:* The large H. V. seen in our case and in others, Engfeldt and coworkers among them, by microradiography, suggests that these peculiar H V. are one of the characteristic pathological facets of the disease. They also add weight to Frost's suggestion that there is a chemical disturbance in osteocyte metabolism in this disease which might have a common chemical denominator with that in the renal tubule. Since the disease is genetically transmitted this is all the more reasonable.

CONCLUSION

The preceding thoughts will, it is hoped, prove stimulating. The existence of H. V. should allow us to turn the tables on the osteocyte. Previously it has remained hidden in its bony shell from our investigative weapons. Now an alteration in H. V. size or pattern should permit us, by using the alteration as an indication of some disturbance in cell metabolism, to turn the obscuring shell against the cell and make it yield useful information.

SUMMARY

Evidence is presented indicating that 0.5 - 2.0 u of the bone wall of the lacunar and canalicular lumens which contain live osteocytes are quasipermeable in the normal, perfectly fresh state. Numerous procedures which are routine in histopathology remove this permeability. Disturbed halo volumes—the designation of the permeable shell by the authors—have been found by the present and previous workers in rats given excessive parathormone and in human vitamin D resistant rickets.

The study of H. V. alterations is expected to implement study of osteocyte metabolism.

REFERENCES

1. Dallemagne, M. J., and Fabry, C.: Acta chir. belg. Supp. 2:75, 1956.

2. Engfeldt, B., Zetterström, R., and Winberg, J.: Primary viatmin-D resistant rickets. III. Biophysical studies of skeletal tissue, J. Bone & Joint Surg. 38A:1323, 1956.

3. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:272, 1958.

4. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

5. Frost, H. M.: Some observations on bone mineral in a case of vitamin D resistant rickets, Henry Ford Hosp. M. Bull. 6:300, 1958.

6. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

7. Frost, H. M.: In vivo osteocyte death. J. Bone & Joint Surg. 42A:138, 1960.

8. Frost H. M.: Biochemical and biophysical characteristics of bone. Read before the Orthopaedic Research Society, Chicago, Ill., January 1957.

9. Heller-Steinberg, M.: Ground substance, bone salts and cellular activity in bone formation and destruction, Am. J. Anat. 89:347, 1951.

10. Jaffe, H. L., and Pomeranz, M. M.: Changes in bones of extremities amputated because of arteriovascular disease, Arch. Surg. 29:566, 1934.

11. McLean, F. C.: Ultrastructure and function of bone, Science 127:451, 1958.

12. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

13. Neuman, W. F., Firschein, H., Chen, P. S., Jr., Mulryan, B. J. and DiStefano, V.: On the mechanism of action of parathormone, J. Am. Chem. Soc. 78:3863, 1956.

14. Sherman, M. S., and Selakovich, W. G.: Bone changes in chronic circulatory insufficiency; histopathological study, J. Bone & Joint Surg. 39A:892, 1957.

, 15. Silberberg, R., and Silberberg, M.: Skeletal effects of radio-iodine induced thyroid deficiency in mice as influenced by sex, age and strain, Am. J. Anat. 95:263, 1954.

MEASUREMENT OF BONE FORMATION IN A 57 YEAR OLD MAN BY MEANS OF TETRACYCLINES*

H. M. Frost, A. R. Villanueva and H. Roth

Until Hiroshima and Nagasaki assumed international importance, the rates and modes of bone turnover in man were primarily academic matters. The advent of atomic weapons, weapons testing, the risks attendant to their manufacture and increasing clinical use of radioisotopes made the physiology of bone turnover in man a practical problem. Research intended to illuminate mechanisms, rates and quantities associated with turnover of bone and bone-seeking substances has been hampered by limitations imposed in using man as a laboratory animal. The immediate and latent risks attending administration of radioisotopes limit the applicability of existing radioanalytical techniques to bone at the light microscopic level. The large and detailed body of knowledge gained from animal work needs qualitative and quantitative corroboration in man before it can be applied to him. As a result of this state of affairs and others undiscussed, we do not have actual measurements of bone-forming activity in man at present. Such measurements are increasingly necessary for work in the fields of radiobiology, osteoporosis of all types, osteomalacia, growth and fracture healing.

This paper amplifies basic work of Milch, Rall and Tobie.[26,27] They noted the fixation of tetracycline antibiotics in mineralizing parts of the skeleton (tetracycline bone labelling) and subsequently detected the drugs by fluorescence in undecalcified bone sections. Our contributions to their work include amplification of the optical behavior of achromycin,[12] an economical method of observing tetracycline fluorescence in bone[13] and illustration with a single human case of the kind of information which tetracycline labelling may yield.

We feel the chief value of this paper is that it presents simple, reliable, effective and precise techniques for measuring bone formation in man in vivo. With these techniques, it should be possible to determine with considerable precision the factors affecting human osteoblastic activity.

Tetracycline bone labelling has the advantages of little immediate and no known latent risk. Numerous opportunities in large medical centers occur to obtain human tetracycline labelled bone from both sexes, all ages and diverse disease states. Use of tetracyclines in human experimentation should be safe.

By virtue of their permanent fixation in mineralizing, new skeletal tissue the tetracyclines label bone forming activity current during the period of drug dosage.[12,17,26,27] Suitable preparation and examination of bones from patients who received tetracyclines at known times and for known duration permits us to measure their bone turnover more than 6 years after tetracycline administration. It is an eerie feeling to realize that one can measure precisely the osteoblastic activity going on in a bone 6 or more years before the patient died![17]

Using tetracycline labelled bone from 43 patients, and with the aid of methods largely developed in the Henry Ford Hospital Orthopaedic Research Laboratory, we

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have been able to measure in man, *in vivo:* the biological half life of Haversian bone; the biological half life of entire bones; rates, quantities and variations in remodelling activity in different bones; osteoblastic activity; and the time required to form Haversian systems.

In this paper the *methods* used for such measurements are outlined through the device of analyzing one human case. Some of the kinds of information that can be wrung from tetracycline labelling are presented for this case. Presentation of detailed measurements of large numbers of human cases, and the effects of disease on bone turnover, will be presented in separate publications.

MATERIALS

The materials studied herein are the middle thirds of the femur, tibia, fibula and entire second metatarsal of the amputated left lower extremity. Corroboratory material for some of the minor points presented in the following paragraphs are sections made as recommended from more than 40 other patients who received tetracyclines before death or surgery.[17] Clinical details of the case are presented in Appendix (A).

METHODS

Four or more complete cross sections of the listed bones were made, stained and mounted by Frost's techniques.[7,8]

Fluorescence: As noted by Milch and coworkers[25,27] refrigeration does not affect tetracycline fluorescence in bone. Material stored in bulk 8 months is unaffected. Thin sections kept in ethanol, however, gradually lose their fluorescence over many weeks. Permanent mounts made as recommended have not faded perceptibly in 3 years. Tetracycline fixed in bone in vivo does fade perceptibly after 6 years of life.[12]

The microfluorescence equipment used by Milch and coworkers is excellent. Fluorescence can also be energized by a 430 mu band in the visual spectrum. A set-up for this method is Wratten 47 and 47B filters in series in the light source and Wratten 8 and 9 filters in series over the eyepiece. The light source should be a high intensity tungsten source such as a 300 watt 35 mm slide projector. Kohler illumination is superior to others with this arrangement.[1] Tetracyclines fixed in tissue fluoresce strongly with this arrangement. Their color is white to gold depending on drug concentration and section thickness. Background is a faint green or magenta. Fluorescence may be proved by passing the Wratten 8 filter under the condenser, whereupon fluorescence abruptly ceases. The condenser should be racked close to the slide and the aperture stop be wide open. For further details consult reference (13). See Figure 2.

Yellow color in bright field Microscopy: Achromycin[a] in achromycin-labelled sections prepared as recommended[7,8] develops a lemon yellow color within one to 14 days of mounting (in Harleco synthetic resin-xylene).[12] Failure to stain and mount as recommended usually prevents this color from appearing. The color does not develop in bulk material. Once developed it appears to be permanent as long as

(a) All of the currently available tetracycline antibiotics, singly or in combination, are nearly equally suitable for work of the type described in this paper.

the mount is preserved. The visible yellow areas correspond precisely to the fluorescent areas. Very thin planes of achromycin labelling, or zones labelled during time of low dosage, may not develop detectable yellow in bright field but will fluoresce.

Appearance of tetracycline labelled bone: Labelled bone appears as yellow-stained bands, the bands being parallel to and in the substance of lamellae. Bands do not cross lamellae or cement lines. The drug deposits in mineralizing bone and cartilage and appears fixed there by subsequent mineralization. No diffusion out of labelled volumes of bone has been seen in mounts 3 years old or in bulk material 8 months old. The labelled band-width is a direct function of *duration* of dosage and *rate* of mineralization of osteoid while intensity of fluorescence and visual yellow is a function of dosage per day. When life continues after cessation of dosage the labelled bone and calcified cartilage become buried by unlabelled bone and calcified cartilage unless appositional growth ceased during drug administration. During the period of tetracycline administration most tissues are stained by the drug. Within 48 hours of drug cessation "adventitious" tetracycline has disappeared from bone, leaving only the labelled bone and calcified cartilage behind as a tell-tale. This and other observations suggest to us that tetracycline staining of bone is ordinarily a readily reversible phenomenon due at least in part to the ability of tetracycline antibiotics to complex with calcium ions. For further details consult references (12, 26, 27).

Permanent staining of Haversian canals, osteocyte lacunae in Haversian systems and calcified cartilage (which were mature during the period of drug administration) has not been seen.[12,17,20]

Observation of the visual yellow in tetracycline labelled undecalcified sections is aided by use of light corrected to 5000°K, apochromats and fully illuminated objectives.[3,28]

Photomicrography: The visual yellow in brightfield is due to fluorescence of tetracycline energized by the blue end of the visible spectrum and so cannot be photographed on ordinary black and white emulsions; excellent color photomicrographs with Ektachrome, and black and white with fluorescence, can be obtained.[4,6] (See Figs. 1 and 2).

MEASUREMENT METHODOLOGY

In order to measure bone turnover in man, we must be able to measure the amount of new bone formed in a unit volume of bone in a unit of time. Tetracyclines administered *in vivo* conveniently label bone actively forming, and conveniently they do not label unmineralized osteoid or already mineralized bone. The tetracycline labelling process supplies the same type of information one would obtain by feeding large amounts of alizarin to man, but without the toxicity associated with alizarin.

In the following material in the *Methodology* section, the actual measurements required are outlined. In the *Data* Section, the manner in which the measurements are manipulated to provide useful information is outlined. We make extensive reference to other publications to shorten an already lengthy paper.

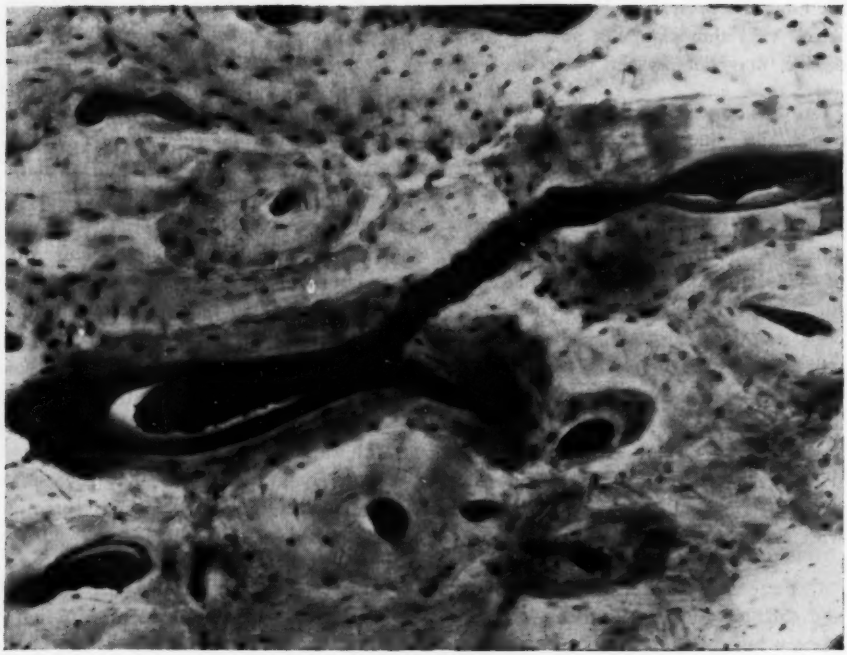

Figure 1

Photomicrograph, Cross Section, femur, basic fuchsin, undecalcified, of the case presented in the text. Brightfield, 200X. The black dots are vascular channels. The dark zone connecting the Haversian channel at left midcenter to the Haversian channel at 2:00 o'clock is a Volkmann's canal which is out of focus. The two Haversian channels are lying in newly formed Haversian systems and are still fuchsin permeable in some areas, causing them to appear darker than the remainder of the bone visible in this illustration. Parts of 3 Haversian systems banded by achromycin and pronouncedly yellow on the original section are in this field but are not demonstrable on the photograph for reasons referred to in the text.

Because of known distributional differences in various parts of single bones, complete cross sections were necessary for the following measurements.[10,11,15,18,19] The longitudinal grain of bone is associated with much less distributional variation along the long axis than is found along the radial and tangential axes. Longitudinal sections also provide too limited a sample. We emphasize here, and will reemphasize later, that the present measurements are done on the diaphyses of 4 bones of one patient and must not be considered representative of norms for the sex and age of all similar people.

The means of measurement used for the data in Charts I and II are now presented.

1. *Channels/mm²:* The slide was marked with a grid of lines which are scanned in increments of one field diameter, using an objective-eyepiece combination of known field diameter. The total numbers of longitudinally coursing vascular channels and of fields are recorded and reduced to channels/mm² by calculation. Relative variation in the present case was small, about 4%. Sampling

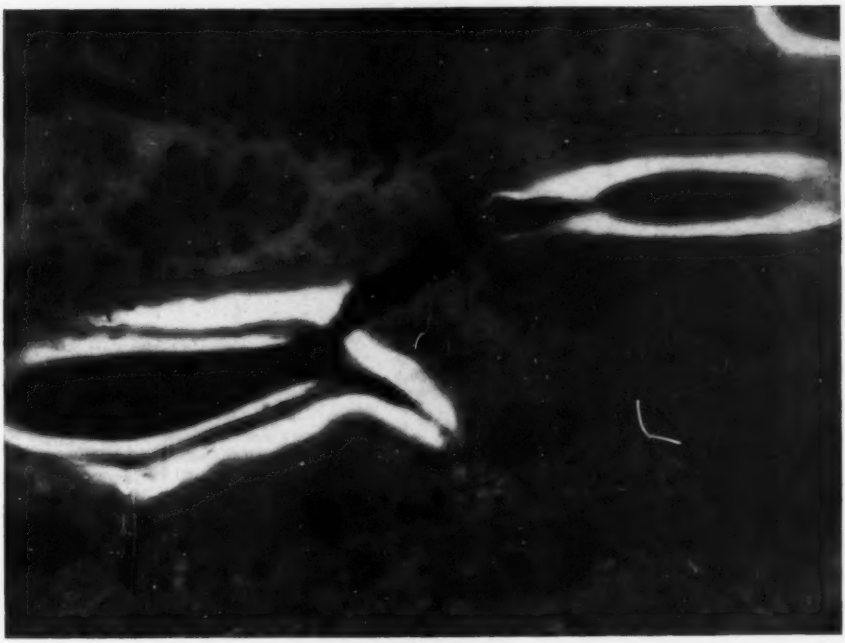

Figure 2

Same field and magnification but with Frost's fluorescence set-up. The bright bands are achromycin labelled parts of the Haversian systems. The left hand system is doubly banded, the outer band being formed during the first, and the inner band during the second achromycin epoch of the case discussed in the text. The right hand Haversian system is singly banded and it is not apparent from the original material whether the band formed during the first or second epoch.

technique must be properly designed or systematic distributional effects inherent in bone and the observer will introduce error. For example there are 2 to 3 times as many channels near periosteal surfaces as near endosteal, while osteon density and incidence of banded osteons is greater near bone angles than in broad bone surfaces. For full details consult references (18, 19).

2. *Percent osteons[a] labelled; labelled osteons per section; percent osteoid seams; percent seams in labelled osteons:* Counts are done as in (1) using a fully illuminated low power apochromat and color corrected light, tabulating counts during the process with the aid of a hand counter. The complete sections, rather than a sample, were scanned for labelled osteons per section, using the mechanical stage to avoid under—or over lap. (Consult references 12, 18, 19, for details of the measurement and for explanation of osteoid seams and seam counts).

3. *Osteons banded in outer, middle, inner thirds or doubly banded; banded osteon lying in outer, middle or inner thirds of cortices:* At any one time

(a) An osteon is an Haversian system.

MEASURED DATA

Chart I

Measured Characteristic	Femur	Tibia	Fibula	Metatarsal
Longitudinal vascular channels/mm²	13.2	14.1	12.2	16.2
% Osteons Banded (Number counted)	2.1 5207	0.65 2000	2.4 1448	1.06 662
Number banded osteons per section, average of 3 or more	149	26	24.5	9
% banded osteons with osteoid seam	4.7	3.8	24.5	11.1
Double banded osteons as % all banded osteons	11.1	11.5		11.1
Outer Third of Osteon Banded	26.1	21.7	35.1	28.6
Middle Third of Osteon Banded	25.8	30.4	25.6	28.6
Inner Third of Osteon Banded	47.0	47.8	38.4	43.3
Unclassifiable	11.0	11.7	20.6	22.2
Banded Osteons Outer Third Cortex	56.3	95.7	84.3	100
Banded Osteons Middle Third Cortex	26.3	4.2	15.6	0
Banded Osteons Inner Third Cortex	17.3	0	0	0

MEASURED DATA

Chart II

Measured Characteristic	Femur
% Osteons Containing Osteoid Seam at Amputation (2100 counted)	0.098
Average Osteon Width	80 u
Range	18.5 - 135 u
Average Width Labelled Bands,	19.9 u
Unselected Range	3.1 - 30 u
Average Width Outer and Middle Third Bands	26.0 u
Average Width Inner Third Bands	6.0 u
% Section Surface Vascular Channel	10%
% Section Surface Banded	.58
% Empty Lacunae, Banded Osteons	2.1
% Empty Lacunae, Inner Third Band of Doubly Banded Osteons	0.5%
% Empty Lacunae, Outer Third Band of Doubly Banded Osteons	2.3%
% Empty Lacunae, Mature Haversian Systems	18.5
% Empty Lacunae, Extra-Haversian	55.7

CALCULATED DATA: SEE TEXT

Chart III

DATA	Femur	Tibia	Fibula	Metatarsal
Osteons/Banded Osteon	45.4	154	41.4	94.3
Banded Osteons/mm²	0.290	0.091	0.294	0.176
Mean Osteon Formation Time	5 wks.	5 wks.	5 wks.	5 wks.
Osteoid Seam Thickness Formed/Day	2.4 u	—	—	—
Instantaneous % Osteoid Seams	0.75	0.23	0.86	0.38
Instantaneous Quiescent Osteons/Mineralizing Osteon	125	430	115	262
Biological Half Life, Entire Cortex				
Years	7.6	24.2	7.6	12.6
Days	2700	8600	2700	4500
Biological Half Life, Osteons				
Years	2.7	8.6	3.1	5.6
Days	960	3000	1100	2000
% Osteons Remodelled per Year	18.7	5.9	21.4	9.4
% Mass (and osseous volume less vascular and cell volume) Mineralizing/Day	0.018	0.0058	0.018	0.011
% Mass in Turnover/Day	0.036	0.012	0.036	0.022
Ratio of Remodelling Activity per Unit Volume (Femur=100)	100	31	101	61
Ratio of Remodelling Activity in Terms Total Mass in Turnover (Femur=100)	100	19	15	7.5
% Remodelling Activity in Outer Third Cortex	56.3	95.7	84.3	100

in a given patient, some osteons will be in the initial stages of formation, some in the final stages and some in between. Labelling with tetracycline will accordingly appear, in the subsequently completed osten, as a band in the outer, middle or inner third of the osteon. If two periods of drug dosage were close enough together, then some osteons would exhibit double bands, one within the other as in Figure 2. Banded osteons were tabulated separately by completely scanning sections of femur, tibia, fibula and mentatarsal with a low power apochromat. 344 banded osteons were counted for these measurements. Doubly banded osteons were counted twice. Of the total banded osteons, 11% could not be classified as to the third of the osteon banded *so these figures do not add up to 100%*.

4. *Average width[a] of osteon; average widths of labelled bands:* Banded osteons were selected at random and measured with a calibrated eye-piece micrometer.[3,28] The totals were reduced to arithmetic means. 49 osteons were measured for average osteon width; 29 unselected bands, 29 outer or middle third bands and 29 inner third bands for average band widths. The average osteon *diameter* is more than twice the listed value since the vascular canal was ignored. The average would be about 240 u with a range of 40-300 u.[17]

5. *Percent empty lacunae:* Reference[11] discusses the technique utilized in these measurements. Briefly lacunae without stained contents are empty and dead. More empty lacunae exist in extra-Haversian than in Haversian bone[b]. The

(a) Width here refers to the distance between cement line and Haversian canal along a radial axis. See Figs. 1 and 2.

inference is that increasing age of patient and bone moiety increases the counts of empty lacunae. Lacunae opening into the section surfaces are not counted. High numerical aperture is essential for the counts.

6. *Percent section surface area:* The theory of this technique is that in a well mixed, multiphase solid, an infinitely thin plane cut through it will contain proportionate surface areas of each phase in direct proportion to the volume components of each phase in the solid, provided the area of the plane is sufficiently large to avoid random distributional effects. This ideal plane may in turn be sampled by running the cross-hairs of a filar micrometer across it, or by other means. Enough runs to reduce random distributional effects below the desired level must be made. Consult references 15, 16, 19, 22, 23, for fuller details.

   The Leitz integrating eyepiece micrometer was designed for the above type of measurement. The microscope objective is used to provide the measured plane, and means for mechanically adding the various phase constituents measured are built into the instrument. The femur was selected for the measurements because of its greater homogeneity as compared to the other bones. For example, the relative variation in counts of total labelled osteons per section was only 0.6% in the femur, 4% in the tibia, 10% in the fibula and 20% in the metatarsal. This suggests that when enough cross section area has been sampled the results are valid for adjacent parts of a diaphysis, and that more sections of small bones must be measured to achieve meaningful results.[19]

   In the present integrating eyepiece measurements, 12 fields were measured at 150 X and 0.32 N.A. with full illumination of the objective. The estimated probable error (a combination of instrument, resolving power, optical, section thickness and operator effects)[3,4,19,21,22,28] is plus or minus 10% for the vascular and 40% for the banded areas.

7. The volume of lacunae and canaliculae is 2.5% of the cortex exclusive of vascular volume. This measurement is detailed in reference 16.

8. Charts I and II list the values obtained for the measurements described above. The data in Chart III are calculated from the data in Charts I and II. See following text for the reasoning involved.

DATA ANALYSIS

1. *Osteon turnover:* Several informative calculations can be made from the data in Charts I and II. These calculations tell us how many osteons are being formed per unit time and per unit volume of diaphyseal bone. They also tell us the proportion of the total osteon population undergoing remodelling per unit time. From this the biological half-life of the osteon population may be caculated for each diaphysis measured.

(b) Haversian bone as defined for the present purpose consists of intact osteons only. In double or triple systems only the innermost one is considered Haversian. The remainder of the cortex is designated extra-Haversian and includes circumferential lamellae and fragments of old osteons.

(A) During the 40 days of drug administration in this case, the percentage of the total number of osteons mineralizing was: femur 2.1%; tibia 0.65%; fibula 2.4%; metatarsal 1.06%.

(B) The number of quiescent (fully formed) osteons per mineralizing osteon were: femur 45.4; tibia 154; fibula 41.1; metatarsal 94.3.

(C) From the vascular channels/mm^2 for each bone the banded osteons/mm^2, thus osteons/mm^2 mineralizing in 40 days, may be calculated: femur 0.29; tibia 0.091; fibula 0.29; metatarsal 0.18. Reasoning elaborated elsewhere reveals that these figures are representative of mineralizing osteons/mm^3 also.

(D) Since 40 days is 0.113 years the proportion of osteons undergoing mineralization in a year can be calculated: femur 18.7%; tibia 5.9%; fibula 21.4%; metatarsal 9.4%.

(E) The biological half-life of osteons may now be estimated. Note that osteons comprise about 0.3 of the total cortices[17] and that a different half-life for the entire cortex will be calculated from direct measurement. Osteon half-lives are: femur 2.7; tibia 8.5; fibula 2.4; metatarsal 5.3, all in years.

(F) Several striking features emerge from the above measurements: the long half-life of osteons, the similarity of the fibula and femur in remodelling activity in this patient, the decrease in remodelling activity as examination proceeds from proximal to distal (suggesting that in the axial skeleton higher rates occur in this patient) and the remarkable inactivity of the tibia. One wonders if the tibial values reflect a general characteristic or if unrecognized disease affected this bone, but not the fibula, in this patient.

It is reasonably assumed that as much bone is broken down as is formed in a given unit time, barring certain pathological states. If this be true then the calculations in (1) should also apply to the rates of bone destruction.

2. *Osteon formation time:* Here we will determine how long, on the average, it took for an osteon to form in this patient in the bones examined. Two separate approaches to the problem are possible in the present case. One is based on the frequency distribution of banded osteons, the other on a simple proportional measurement of width of the bands and width of the osteons.

(A) About 11% of the total banded osteons were doubly banded, first in the outer, then in the inner third of the osteon's width. See Figure 2. The outer bands average 20 u, the inner bands 6 u thick. A mean time interval between labelling epochs of 7 weeks will be assumed. This is the time during which all of the doubly banded osteons formed. If this were the *mean* time for *all* osteons there should be about as

many that formed faster than 7 weeks as formed slower. The faster ones would exhibit one band but the slower ones would exhibit two bands, *one of which was in the middle third of the osteon.* Osteons singly banded in either middle or outer thirds constitute about 40% of the *total* but there were only 3 doubly banded osteons one of whose bands was in the middle third, in 344 counted. This suggests that the *mean osteon formation time* is less than 7 weeks. A period of about 5 weeks will be assumed because of the following reasoning.

(B) The average osteon width was 80 u. The average width of the middle and outer third achromycin bands in doubly banded osteons was 26 u. These bands were labelled during a drug administration time of 11 days. 80/26 X 11 should be the time of osteon formation. This is 34 days. It is not certain that ignoring the first 3 days of drug administration is justified or that the rate of osteon formation is steady, however, matters which we have assumed to be the case for the moment. Nevertheless the evidence is compatible with an osteon formation time of about 5 weeks in this patient.

(C) Assuming 5 weeks to be correct, 2.4 u of osteoid a day were elaborated in mineralizing osteons in this patient.

3. *Instantaneous percentage of osteoid seams;*[9,18,19] This is the percent longitudinal vascular channels containing mineralizing bone and thus a seam at any one moment. It is assumed here that the percentage of osteoid seams and the percentage of labelled osteons are equivalent. Subsequent publications will document this assumption.

(A) Assume osteon formation rate and numbers were equal during the first and initial part of the second drug administration periods. Then half the singly banded osteons were labelled in the first 11 day period. During 11 days some osteons completed, while others commenced, formation, causing some overlap. The overlap should be about 11/35 of the instantaneous value. On this basis the value of osteons forming at any one moment for the femur is 0.75%. The value found at amputation was 0.1%. This indicates that the vascular events preceding gangrene markedly retarded new osteoid formation. The reader should note that the somewhat unrealistic assumptions made here are *unfavorable* to the qualitative result derived. The retardation in bone forming rate which occurred during the second achromycin administration epoch was probably *greater* by about 40% than the figure we quote.

(B) Using a conversion factor of 0.36[a], the incidence of banded osteons can be converted to supply the instantaneous value of osteoid seams. Chart III lists them.

4. *Turnover of entire diaphysis:* Because there are marked variations in the proportions of Haversian to extra-Haversian bone in various bones and in

(a) 11 days drug dosage÷35 days osteon formation time.

various ages, determination of osteon turnover or half-life does not tell us about turnover or half-life of the entire bone cross-section. Tetracycline labelling permits a direct measurement of entire cross-section turnover to be made by planimetric means.

(A) A representative femoral cross-section was measured with the Leitz integrating eyepiece micrometer. 10% of the area was vascular channel and 0.58% was banded by achromycin. The volume of osteocytes and canaliculae is roughly 2%.

Thus while 0.58% of the total cortical volume was banded, 0.63% of the dry, fat-free cortical mass was banded, assuming uniform bone density, by 40 days of achromycin dosage.[b]

We calculate that 0.018% of this femur was mineralized through new bone formation per day. According to previous reasoning this is also the amount undergoing destruction per day. If the patient's vascular difficulties retarded bone formation during the second achromycin dosage period, this figure and those below are too small by a factor of, at most, 40%.

(B) If the percent of mass undergoing mineralization per day is dependent on the percentage of banded osteons, estimates of mineralizing mass per day can be calculated for the other bones by simple proportion. Values are: tibia 0.0058%; fibula 0.018%; metatarsal 0.011%.

(C) Bone turnover as utilized here means the sum total of bone being formed and degraded. This is twice the formation quantity. The values per day: femur 0.036%; tibia 0.012%; fibula 0.036%; metatarsal 0.022%. These are precent of mass, or percent of osseous volume excluding vascular and cellular volume. These measurements assume a normal balance between bone formation and destruction, something probably not present in the particular case we present. Another, direct means of measuring bone resorption quantity and rate is needed to evaluate bone turnover in disease states such as osteomalacia and senile osteoporosis.

(D) The remodelling rates and quantities of the various bones may now be compared with each other. In terms of mass per unit volume turned over, with the femur set arbitrarily at 100, the values are: femur 100; tibia 19; fibula 101; metatarsal 61.

In terms of the absolute mass turning over in the middle thirds of the bones, using the banded osteons per complete cross-section as a means of proportional comparison, and with the femur set arbitrarily at 100, the values are: femur 100; tibia 19; fibula 15; metatarsal 7.5. In these calculations we assume there is no significant difference in the

(b) Bone density is not uniform at the macroscopic or microscopic levels but it is permissible at present to assume a uniform average density. For some work on density variations consult references 29, 31.

size of osteons in the various bones. If high precision is required, this assumption would require documentation by measurements on the separate bones.

(E) The mean half-life of the entire cortex may now be calculated, using percent of mass mineralizing per day as the base. The values are, in years: femur 7.6; tibia 24.2; fibula 7.6; metatarsal 12.6.

(F) It will be noted that the osteon half-life is about 0.3 the above values. This suggests that there are different turnover rates for Haversian and extra-Haversian bone.

This inference is substantiated by finding appreciable portions of circumferential lamellae in extra-Haversian bone even in very old patients. Since circumferential lamellae form during appositional growth in all phases of childhood, to be later partially replaced by osteons, it is probable that the circumferential lamellae in this patient's extra-Haversian bone is older than the half-life values suggest, in some instances probably 40 years old. It may be noted here that micropetrosis affects extra-Haversian bone, particularly circumferential lamellae, long before it occurs in Haversian bone. Micropetrosis is related to the biological age of the bone affected and is not found in young bone.[11] These points also suggest a much longer half-life for extra-Haversian than for Haversian bone.

5. *Locus of remodelling activity:* The distribution of banded osteons in the cortical thirds is inhomogeneous. Most of the remodelling activity occurs in the outer third of the cortex. In the femur, correction for the difference in vascular channels/mm² between periosteal and endosteal surfaces balances the difference out, but this correction does not balance out the preference for outer third remodelling activity in the rest of the bones. These facts pose two questions: why should remodelling activity concentrate near the periosteal surfaces, and why are there more vascular channels there than elsewhere? While a peculiarity of periosteal blood supply might be postulated, it is also possible that the greater surface stresses and strains play a role. This is a problem which should prove amenable to experiment using tetracycline labelling as an investigative method.

6. The case we present offers an opportunity to check the thesis that one of the factors causing bone cells to die in vivo[10] is age. We performed counts of percent empty lacunae in this case which reveal:

Banded osteons (known to be younger than 95 days) had an average of 2.1% empty lacunae.

The inner band of doubly banded osteons had 0.5%; the outer band 2.3% empty lacunae. (Larger differences in empty lacunae between central and peripheral lacunae usually occur in human Haversian systems so this is a poor index of how many cells might die in 95 days).

Mature, normally mineralized osteons exhibit an average of 18.5% empty lacunae.

Extra-Haversian bone exhibits an average of 55.7% empty lacunae, but with variations. Micropetrotic bone for example comprises about 10% of the femur (on which these counts were done) but is 100% dead. It is noteworthy that no banded micropetrotic areas were seen in any of the 20 sections scanned for this purpose or in any of the 42 other cases available to us which are labelled in vivo with tetracyclines.

It appears that the empty-lacuna counting technique might serve as a means of assigning relative ages to various parts of the same bone, extraneous factors being absent. In this case a definite correlation between age of the bone and percent empty lacunae exists.

DISCUSSION

A.) We wish to emphasize that the foregoing measurements and calculations are valid only for the diaphyses of the bones of the case examined. Extrapolations to the whole bone or skeleton or to man as a whole are not justified. There is reason to expect that in the future factors of equivalence may be determined which will permit such extrapolation.[5] Such factors would prove invaluable in skeletal research and clinical work.

B.) The behavior of tetracyclines is not necessarily representative of the behavior of all bone-seeking substances. Differences in ionic size, sterism, charge, solubility, dielectric constant, chemistry, hydration shells and other factors make such similarity unlikely. Nevertheless it is possible in some instances that equivalence factors are determinable which would permit the behavior of tetracyclines and some other hypothetical substance to be equated. Some likely candidates, with the qualifications below in mind, are lead, strontium, radium and calcium.

As an example of how misleading the figures on the amount of bone labelled per day by achromycin may be, consider the following: Partly published work[8,9,10] reveals that in mineralizing osteons containing an osteoid seam there is a thick zone of permeability beyond the seam to diverse ions and large molecules. The zone thickness usually exceeds 15 u and represents the instantaneous volume of the mineralizing osteon which is readily permeable to these substances. Achromycin, however, deposits only in a thin band at the periphery of the seam,[12] affecting a volume of bone smaller by a factor of about 20. Such a difference in behavior reqiures explanation and makes one cautious about assuming permeability similarities in two different substances.

C.) This paper deals with only one aspect of bone turnover as that term is commonly understood, and that is bone formation and bone destruction. These are not the only mechanisms by which bone-seeking substances may enter or leave the skeleton, and therefore not the only important mechanisms involved in radiobiological turnover and exchange work with bone. It is now possible, however, to assign turnover, in the sense used by us, some

quantitative meaning so that other modes of turnover may be placed in perspective.

D.) The techniques which we have utilized in this work, and the manner in which we have applied these techniques, should pave the way for accurate measurement of normal osteoblastic activity in man. This is because the newly mineralizing bone labelled by tetracyclines must be formed by osteoblasts, and measuring the product is equivalent to measuring the production rate directly.

E.) With the above means it should be possible to determine what and how various factors affect osteoblastic activity. For example, do estrogens really accelerate osteoblastic activity in man, or do they exert a different effect? How do the corticoids, thyroxin and growth hormone act and what is their relative importance? These are some of the questions we hope to be able to answer in the future. It is astonishing how little hard, factual knowledge exists at present and serves as a basis for a large edifice of clinical theory concerning osteoblastic activity.[1,2]

F.) When the factors affecting osteoblastic activity in man have been recognized through work on the lines suggested above, the clinician will be able to *cure* osteoporosis of any type, to affect bone growth in any desired manner and to cause fractures to heal more dependably and in less time than is currently required. Much work, of course, lies between us and such capabilities.

G.) The bone formation studied in the present case was *lamellar* bone formation. It has been pointed out elsewhere that fibrous and lamellar bone osteoblastic activity differ in some manner in their chemical processes and in their regulatory mechanisms.[14] In past experimental work on osteoblastic activity, failure to distinguish between these two fundamental bone types necessarily invalidates the interpretation of such research.[1,2]

SUMMARY

In vivo tetracycline labelling of bones from one patient, from which undecalcified sections of the femur, tibia, fibula and metatarsal were made, permitted the *direct* measurement or estimation in a 57 year old man of the following aspects of bone physiology:

Osteon turnover rate and half-life in the various bones examined.

Total diaphyseal bone turnover rate and half-life in the various bones examined.

Mean time of osteon formation.

Locus of the major part of bone remodelling activity.

Comparison of turnover rates in different bones.

Quantitation of bone formation and destruction per unit time and volume in the bones examined.

The techniques used in making the above determinations offer new means of studying human bone physiology. Within their limitations of scope, they should prove

useful in advancing our knowledge of skeletal dynamics.

The data presented in this paper are the first time such direct measurements have been made in man.

APPENDEX (A)

CASE PRESENTATION

Briefly, a 57 year old Caucasian male developed an aortic aneurysm which was resected and replaced with a prosthesis. An interval of good health followed. Then infection and leakage, after several temporizing surgical procedures, led to complete ligation of the blood supply to the left lower extremity, gangrene and pertrochanteric amputation. These events occurred during two separate hospitalizations in each of which the patient received known amounts of achromycin for known time periods.

There were no objective or subjective circulatory difficulties in his lower extremities until a thrombophlebitis of 5 days duration appeared on the 48th day after first achromycin administration. Transient circulatory difficulty appeared on the 75th day after a surgical procedure. Gangrene occurred on the 97th day after ligation of all collaterals to the limb and extirpation of the entire left limb of the arterial prosthesis. Amputation was done on the 102nd day. The amputated limb provided the material for the present study.

Achromycin was administered in dosage of 2.0 gm/day on the following days, numbering them from the day of first administration 1-3; 11-21; 63-88. No achromycin was given on the other days. These periods will be referred to subsequently as the first and second achromycin epochs, days 1-3 being ignored.

Multiple other antibiotics were administered but no other tetracyclines. (All of the other antibiotics and the tetracyclines have been tested in vitro for bone staining; only the tetracyclines fluoresce and stain undecalcified bone visibly.)

REFERENCES

1. Albright, F., Bloomberg, E., and Smith, P. H.: Post-menopausal osteoporosis, Tr. A. Am. Physicians 55:298, 1940.

2. Albright, F.: Osteoporosis, Ann. Int. Med. 27:861, 1947.

3. Allen, R. M.: The Microscope, New York, D. Van Nostrand, 1940.

4. Allen, R. M.: Photomicrography, New York, D. Van Nostrand, 1941.

5. Eckelmann, W. R., Kulp, J. L., and Schulert, A. R.: Strontium-In man, II. Science 127:266, 1958.

6. Eastman Kodak Co.: Photomicrography, ed. 13, Rochester, New York, 1935.

7. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:272, 1958.

8. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

9. Frost, H. M.: Some observations on bone mineral in a case of vitamin D resistant rickets, Henry Ford Hosp. M. Bull. 6:300, 1958.

10. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

11. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

12. Frost, H. M.: In vivo bone staining with tetracyclines, Stain Technol. 35:135, 1960.

13. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

14. Frost, H. M.: Observations on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1960.

15. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

16. Frost, H. M.: Measurement of osteocytes per unit volume and volume components of osteocytes and canaliculae in man, Henry Ford Hosp. M. Bull. 8:208, 1960.

17. Frost, H. M.: Unpublished work.

18. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

19. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. (In press).,

20. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:255, 1960.

21. Hardy, A. C., and Perrin, F. H.: Principles of Optics, New York, McGraw-Hill, 1932.

22. Hennig, A.: Measuring and counting in microscopy, Zeiss Werkzeitschrift No. 30, Dec. 1958.

23. Hennig, A.: Das Problem der Kernmessung. Eine Zusammenfassung und Erweiterung der Mikroskopischen Messtechnik, Mikroskopie 12:174, 1957.

24. Jaffe, H. L., and Pomeranz, M. M.: Changes in bones of extremities amputated because of arteriovascular disease, Arch. Surg. 29:566, 1934.

25. Jenkins, F. A., and White, H. E.: Fundamentals of Optics, ed. 2, New York, McGraw-Hill, 1950.

26. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

27. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.

28. Needham, G. H.: Practical Use of the Microscope, Including Photomicrography, Springfield, Thomas, 1958.

29. Robinson, R. A., and Elliott, S. R.: Water content of bone. I. Mass of water, inorganic crystals, organic matrix, and "CO_2 Space" components in a unit volume of dog bone, J. Bone & Joint Surg. 39A:167, 1957.

30. Sherman, M. S., and Selakovich, W. G.: Bone changes in chronic circulatory insufficiency; histopathological study, J. Bone & Joint Surg. 39A:892, 1957.

31. Trotter, M., and Peterson, R. R.: Ash weight of human skeletons in per cent of their dry, fat-free weight, Anat. Rec. 123:341, 1955.

PYOGENIC OSTEOMYELITIS: DIFFUSION IN LIVE AND DEAD BONE WITH PARTICULAR REFERENCE TO THE TETRACYCLINE ANTIBIOTICS*

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Short, intensive courses of antibiotic therapy enjoy considerable success in the treatment of soft tissue pyogenic infections. It has been learned empirically that such therapy is seldom adequate in the treatment of pyogenic osteomyelitis, which requires higher doses and longer treatment to reduce recurrences to acceptable levels.[2,5,7] The reasons for these differences are not well known and as a result the advent of each new antibiotic is accompanied by sporadic, unsuccessful attempts to cut short the antibiotic treatment time in cases of pyogenic osteomyelitis. It has been pointed out previously that a bacterial reservoir exists in the one million-plus spaces present in each cubic millimeter of infected bone.[17] This paper considers diffusion processes which affect the ability of administered antibiotics to reach the bacterial reservoir of pyogenic osteomyelitis.[a]

At present it is not possible to treat diffusion process in bone mathematically or even with experimental precision. The reasons for this state of affairs become apparent when the list of factors known to affect diffusion in ideal systems is reviewed. This paper accordingly will present and interpret the results of some *qualitative* experimental work done on living and dead human bone. The tetracycline antibiotics are at the core of our experimental methods because of their convenience, their fixation in bone in vivo,[16] the availability of human bone specimens from a sizable number of patients who received the drugs during life and because of the ease with which they may be detected in very minute amounts by available methods. The methods evolved in this laboratory have the virtue of simplicity and economy as well as the sensitivity of other methods.[15,16]

In order to tell whether diffusion behavior of the tetracycline antibiotics is a special case or if their behavior is generically representative a group of other reagents have been compared to the tetracyclines in bone in vitro. The results of such experiments are summarized. We feel that, in the absence of suitable accurate measuring methods, a detailed presentation of our experiments would constitute needless verbosity. Our data are based on qualitative, visual observations.

FACTORS AFFECTING DIFFUSION IN ISOLATED SYSTEMS[3,6,8,19,20,24]

We are concerned with the diffusion of solute molecules in the extracellular fluids in the spaces of human bone. It has been pointed out elsewhere that diffusion through mineralized bone matrix does not occur in molecules exceeding the size of glucose or similar organic substances. If we consider *diffusion impedence* to be resistance to motion of a solute particle from one point to another in the solvent, we may then list factors known to *increase* diffusion impedence, first in a limitless volume

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(a) The bacterial reservoir is the bacteria harbored in the spaces (lacunae, canaliculae, Haversian and Volkmann's canals and medullary cavity) found in bone.

of solvent, and second along a finite column of solvent bounded by a container whose walls may exhibit specific effects on diffusion impedence.

In a limitless volume of solvent, diffusion impedence is increased by: increase in pressure; decrease in temperature, solute concentration, mean free path; increase in mass and charge of solute and solvent ions; specific interaction effects of one solvent molecule with another, one solute molecule with another and of solvent with solute; by adventitious solutes; by specific effects of pH on solvent and solute; by increase in size of solute and solvent molecules.

In tubular lumens diffusion impedence is increased by: decreasing the radius of the tube lumen (canalicular radius is 200 millimicrons) increasing the length of path from one point to another (as occurs in tortuous tubes); decreasing the total cross section area through which diffusion occurs (about 8 square microns of canalicula connect each lacuna to a vascular channel while the average cross section area of an Haversian canal is 0.005 mm^2); interaction between the wall of the lumen on the one hand and the solutes and solvent on the other; specific effects of pH, dielectric constant of the solvent, solute and wall of the lumen; and finally by the presence of a gel state (a specific interaction effect normally present in biological systems) filling the lumen in which diffusion occurs. This listing is not complete.

It may be seen that quantitatively this is a hopeless problem for the present!

DIFFUSION IN BONE *IN VITRO*

The sum of the following material is that bone is highly impermeable and that diffusion processes in its fluid-filled spaces are normally highly impeded. The impedence "wall" is normally surmounted by energy supplied by living, metabolizing cells.

Our in vitro work was done on fresh human bone. By fresh we mean the bone was obtained and sectioned less than 6 hours after death or surgery. Bones investigated include rib, clavicle, femur, tibia, fibula, radius and ulna. There were no consistent differences among them. The sections referred to subsequently were undecalcified, unfixed, unembedded, undehydrated, thin sections made by Frost's method.[9,10] This method introduces less artifact into bone than any other existing method. Lo Grippo and Hirsch have kept human biopsy specimens prepared by this method alive in special media for periods as long as 6 weeks.[21]

We soaked fresh sections 60 - 70 microns thick in the following reagents: aureomycin, achromycin, terramycin, Ag^{+}, Hg^{++}, Pb^{++}, Cu^{++}, Co^{++}, Fe^{++}, MnO_2, $Cr_2O_7^{--}$, I^{-}, F^{-}, Br^{-}, glucose, urea, various dyes such as acid and basic fuchsin, methylene blue, gentian and crystal violet, toluidine blue, eosin G and Y, saffranin, several haematoxylins, chlorazol black, nile blue sulphate, Sudan III and IV and india ink. Inorganic substances were subsequently demonstrated as colored inorganic precipitates. The dyes were directly visible in situ. Reagents such as glucose, urea and citrate were added to other reagents or used before them to detect blockade, diffusion impedence or bone diffusion phenomena of specific nature.

In aqueous solutions the majority of the above reagents are very poor permeators

of the canaliculae and lacunae in the center of the bone sections over time periods of 2 - 7 days at physiological temperatures and pressures. Concentrations were on the order of 0.1 Molar, except where solubility considerations prohibited. Where it could be investigated the effct of pH over the range of 6.5 - 8.5 was tested with no effect on diffusion impedence noted. Acetate, citrate, carbonate and phosphate buffer systems were used. The specific ion effects need not be considered here. It must be noted that our methods are crude and only rule out a major pH effect on diffusion impedence. It would be astonishing if pH had no effect at all.

We could improve permeation radically by the following: drying sections 4 hours at 40° C before staining, use of 40% ethanolic solutions, and boiling in the reagent. Boiling produces severe, irreversible permeability defects which the other two methods do not.

It is known in life that some osteocyte lacunae are normally empty, the percentage of empty lacunae increasing with age;[12] that in most elderly patients a peculiar obliteration of canaliculae and lacunae occurs which is termed micropetrosis and may affect little or much of the skeleton;[11] and that a peculiar state of incomplete mineralization termed Feathering occurs in human bone, in some cases affecting more than half of the axial skeletal volume.[13] It has been pointed out elsewhere that a molecular size effect can be seen in bone which limits the size of molecules which can diffuse into the bone matrix and which might be expected to affect the exchange of substances between bone and blood.[14]

Our experiments offered us the opportunity to observe diffusion impedence phenomena in the disease states noted above.

In *in vitro* sections there is little difference in permeation of the lacunae and canaliculae between live and dead ones, although the live ones may have slightly better permeation. No diffusion into the canaliculae or matrix of micropetrotic bone has been observed in an extensive amount of material. Ready diffusion into feathered bone occurs.

A molecular size effect on diffusion impedence is clearly present. There are also erratic specific effects of unknown nature which will make a particular substance peculiar in its behavior—methylene blue for instance, a good permeator even in aqueous solutions; and ferric ion which even in dilute solutions decalcifies sections rather than permeates them.

No difficulty was experienced in staining the walls of the vascular channels in the sections used in the above work. We interpret this to mean lower diffusion impedence in these relatively large spaces. The ease of staining the walls of the vascular channels also indicates that the locus of highest diffusion impedence lies in the canaliculae, since these canaliculae open directly into the vascular channels.

When diffusion impedence is checked in fresh, bulk bone* it is found that penetration of all the vascular channels requires over 6 weeks even for moderately thin bones such as ribs, and that this can only be achieved with the use of ethanolic solutions. The job is hopeless with purely aqueous solutions.

*Cubes 1-2 cm. on a side.

We secured experimental evidence that increasing reagent concentration, increasing temperature, using smaller molecules and destroying the gel plug (which bacterial invasion of infarcted bone presumably also does) in vascular channels considerably lowered diffusion impedence.

DIFFUSION IN BONE *IN VIVO*

For these observations we are dependent upon the behavior of tetracyclines administered in vivo over variable periods of time but, in all the cases whose findings are quoted herein, administered continuously up to within a day of surgery or the time of death. Since the tetracycline antibiotics become permanently fixed only in mineralizing bone, and wash out of other tissues when dosage is stopped, the continuance of dosage to within 24 hours of death was a necessary requirement for the present observations.

Tetracyclines were detectable by microfluorescence methods in many but not all lacunae and canaliculae containing live cells after 2-5 days of continuous dosage, but not in the case of shorter dosage. Tetracyclines were usually not detectable in these spaces if the drug had been stopped 48 or more hours before death or surgery, the exception being an anuric, uremic patient who still had demonstrable tetracycline in lacunae 5 days after drug dosage was stopped.

Tetracyclines were not detectable in dead canaliculae or lacunae even after 2 weeks of continuous drug dosage, nor were they detectable in micropetrotic bone.

Tetracyclines were detectable in feathered bone in less than 10% of the feathered systems examined, indicating that some unapparent factors reduce the permeability of feathered bone in vivo.

Tetracyclines were detectable as stains of the walls of the vascular channels as expected but with an unexpected feature: a variable number of the channels were unstained in each section examined. We interpret this to mean that these channels did not contain patent blood supply during the period of drug administration. (In vitro *any* exposed bone surface can be stained with tetracyclines; the surface stain may also be removed at will).

DISCUSSION

General Aspects

Physical-chemical effects have to be invoked to explain the poor diffusivity of the reagents tested in human bone in vitro and in vivo. Effects on diffusion impedence of temperature, concentration, the size and structure of canaliculae, the protoplasmic plug in vascular channels, and size of the diffusing molecules have been observed. So many additional factors might affect diffusion impedence in vivo that we must consider the surface of the topic barely scratched. One outstanding fact emerges from our work, however, and this is the very high diffusion impedence normally found in dead bone, whether in vitro or in vivo.

This diffusion impedence is so high that the histologist may well ask how osteocytes can live in their bony prison. The explanation must be the metabolic pump.[14]

This pump is the system of concentration gradients produced and maintained by the chemical activity of the osteocytes. It is known that cells can concentrate (another way of saying create a concentration gradient) substances by factors of 200 to 10,000,000 and there is no reason to expect osteocytes to be exceptional in this respect.

We have obtained clear evidence that the diffusion of tetracycline antibiotics in dead bone is highly impeded. The similarity of a group of substances chemically unrelated to the tetracyclines makes it probable that high diffusion impedence affects other antibiotics used in the therapy of pyogenic osteomyelitis. Diffusion impedence is greatest in canaliculae. The failure of reagents to diffuse 100 microns along canaliculae in 48 hours or more indicates that some specific effects are present which create a diffusion barrier in the canaliculae.

The in vitro and in vivo impermeability of micropetrotic bone might have been anticipated in view of the nature of this bony change. The failure of most of the feathered bone present in a skeleton to stain with tetracycline in vivo is surprising in view of the ready permeability of feathered bone in vitro. One wonders if feathered bone is "available" to the blood for exchange of the much smaller, more mobile electrolytes and buffer ions. It is possible that some peculiar attribute of feathered bone or its cells makes it less active physiologically than normal bone.

PYOGENIC OSTEOMYELITIS

A bacterial reservoir occurs in osteomyelitic bone.[17] The bacterial reservoir is composed of bacteria harbored in the myriad spaces normally found in bone. These bacteria in some cases are able to remain in viable, potentially infective states for many years and are the usual source of infecting bacteria in a recurrence of pyogenic osteomyelitis.

Diffusion behavior probably explains the failure of short periods of antibiotic treatment to kill all the bacteria in the osteomyelitic bacterial reservoir. The high diffusion impedence to large molecules found in dead bone in vitro and in vivo prevents antibiotic permeation of dead, infected bone in short time periods.

From general knowledge of the pathology of pyogenic osteomyelitis[1,4,18,25] and the work presented here it is possible to explain some aspects of the disease.

First, small portions of dead bone surrounded by good blood supply might be sterilized in 6 to 8 weeks by the proper dosage of antibiotic. The larger the portions of dead bone become, however, the less complete will their permeation by the antibiotic be and the more likely it is that viable bacteria will remain in some of the bone spaces after discontinuance of the antibiotic. Large bone portions, such as the entire cortical thickness over a length of several centimeters of tibia, probably could not be thoroughly permeated with antibiotic even after a year of continuous dosage. This behavior explains the high recurrence rate in cases of pyogenic osteomyelitis in which for any reason institution of proper therapy was delayed. It is in just such cases that large portions of cortical bone die, due mostly to the effects of vascular thrombosis and increased intramedullary pressure.

Second, it should take several days of therapy to ensure life of infected but still living cells. These cells would die if the infecting organism were not subdued

Third, the desired rapid improvement in clinical signs and symptoms which follows administration of antibiotic cannot mean the obliteration of the bacterial reservoir. It means obliteration of the organisms in infected but viable soft tissue and bone. *The bacterial reservoir is a separate problem and its elimination necessitates long, high dosage therapy.*

The failure of antibiotics to sterilize large masses of infected bone gives experimental teeth to the clinical dictum that treatment should be begun when the disease is suspected. Waiting will result in death of larger and larger masses of bone.

Several things may be inferred from our work which may be summarized in the general statement that *it should be possible to tailor the physical chemistry and ordinary chemistry of an antibiotic to the treatment of pyogenic osteomyelitis.* Several attributes of such an antibiotic may be suspected in advance (due to the complexity of the biological system we are dealing with, these attributes must be tested in that system before they are accepted as valid).

Figure 1

Undecalcified cross section clavicle between crossed polarizers, revealing Haversian canals (large black ovals), Haversian systems surrounding the canals, and extrahaversian bone filling in around the Haversian systems. The channel at 6 o'clock is the end of a Volkmann's canal.
The Haversian vessels lie in the Haversian canals. These vessels are the normal source of substances brought to bone by the blood. To permeate the bone a substance must diffuse out of the vessels into the perivascular fluids in the Haversian canal: thence into the canaliculae and thence to the lacunae.

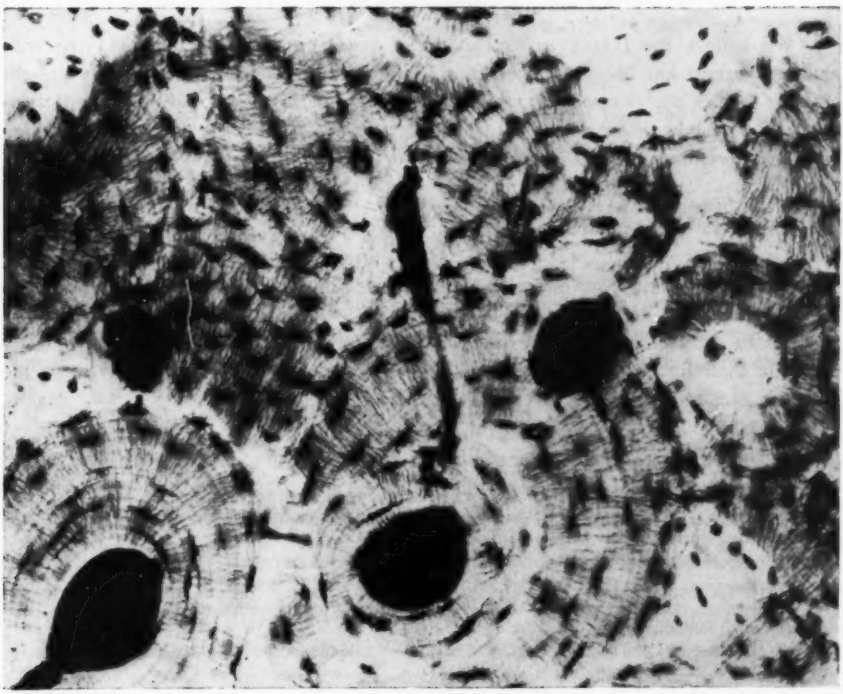

Figure 2

Undecalcified cross section femur, stained with basic fuchsin. Magnification and field selected to reveal the lacunae and their canalicular network. The function of canaliculae is to provide tubular lumens in the impermeable mineralized matrix connecting lacunae to sources of blood supply: Haversian canals.

First, an effective bone permeator should be of small molecular size. Second, it should have small net charge at the pH of the fluids in the bone spaces (this pH is not known). Third, and most promising, one or more specific interaction effects might be used to accelerate permeation of dead bone by the antibiotic. For example, one can speculate that the ability of the tetracyclines to complex with magnesium and calcium might be put to good use.

Theoretically an increase in pH of the body fluids bathing a child's dead bone might increase the rate of accretion of mineral in the dead bone.* If antibiotic molecules could be carried into the dead bone by this slow current of mineral a specific jumping mechanism surmounting the diffusion impedence barrier would be present. We hope the reader will excuse the details of our speculation and instead fasten on the possibility we are discussing: that antibiotics might be "tailored" chemically and physically for the task of treating ostemyelitis.

*Children's bones are on the whole less densely mineralized than adult's bones and are continually accumulating new mineral, although at slow rates. Mineral accretion is more rapid at more alkaline pH.

CONCLUSION

We believe the failure to sterilize large masses of infected bone in vivo with antibiotic agents has been explained by the high diffusion impedence normally present in bone as has the necessity for early diagnosis and treatment and prolonged treatment of pyogenic osteomyelitis. More important, we believe the work and thinking we have presented provides fertile ground for additional work on the several topics touched on in our presentation.

SUMMARY

Both in vitro and in vivo, dead bone is afflicted by a very high diffusion impedence which impairs the permeation of its myriads of tiny spaces by antibiotics. This impedence to antibiotic diffusion explains the failure of courses of antibiotic to cure pyogenic osteomyelitis when given for short periods of time or when given in the presence of large masses of already dead bone.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases, Philadelphia, Saunders, 1958.

2. Altemeier, W. A., and Wadsworth, C. L.: Evaluation of penicillin therapy in acute hematogenous osteomyelitis, J. Bone & Joint Surg. 30A:657, 1948.

3. American Institute of Physics: Handbook, ed. by D. E. Gray, New York, McGraw-Hill, 1957.

4. Anderson, W. A. D.: Pathology, ed. 2, St. Louis, Mosby, 1953.

5. Beerman, C. A.: Treatment of acute hematogenous osteomyelitis of long bones in infants and children, J. Pediat. 33:578, 1948.

6. Goldacre, R. J.: Folding and unfolding of protein molecules as a basis of osmotic work, Internat. Rev. Cytol. 1:135, 1952.

7. Buchman, J.: Acute hematogenous osteomyelitis, Instruct. Course Lect. Am. Acad. Orthop. Surgeons 9:413, 1952.

8. Clark, W. M.: Topics in Physical Chemistry, ed. 2, Baltimore, Williams & Wilkins, 1952.

9. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

10. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

11. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

12. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

13. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

14. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

15. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

16. Frost, H. M.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

17. Frost, H. M.: Pyogenic osteomyelitis: The normal bone spaces as a bacterial reservoir, Henry Ford Hosp. M. Bull. 8:263, 1960.

18. Howorth, M. B.: Textbook of Orthopedics, Philadelphia, Saunders, 1952.

19. Jacobs, M. H.: Diffusion processes, Ergebn. Physiol. 12:1, 1935.

20. Johnson, F. H., Eyring, H., and Polissar, M. J.: Kinetic Basis of Molecular Biology, New York, John Wiley, 1954, Chap. 14.

21. LoGrippo, G., Hirsch, L. J., and Frost, H. M.: Unpublished work.

22. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 6, Philadelphia, Saunders, 1952.

23. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

24. Randall, J. E.: Elements of Biophysics, Chicago, Year Book Publishers, 1958.

25. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.

PYOGENIC OSTEOMYELITIS: THE NORMAL BONE SPACES AS A BACTERIAL RESERVOIR

HAROLD M. FROST, M.D.

It is well known that pyogenic osteomyelitis is a different clinical infectious process from pyogenic soft tissue infections even though the organisms causing the two may be the same. Empirically clincians have learned that, depending on the causative organism, pyogenic osteomyelitis has a significant tendency to recur after cessation of therapy and that larger and longer dosage of antibiotic is necessary in pyogenic bone infections than in pyogenic soft tissue infections.

The reasons for these differences are not generally known or published although the basic factual material needed to explain them is available. In this paper, explanation is begun by outlining the ability of bone's normal spaces to harbor bacteria.

TYPES OF SPACES IN BONE

The gross appearance of a bone is so convincingly that of a homogeneous solid that this concept unconsciously colors our thinking when we deal with skeletal anatomy and physiology in clinical practice. Yet we know that various spaces exist in bone.

The normal spaces in bone are the lacunae which contain the bone cell, the osteocyte; the canaliculae, which connect the lacunae to the nearest source of blood supply, either directly or by a relay system through anastomoses with other lacunae; Haversian canals, which are longitudinal channels containing blood vessels of varying size along with some loose connective tissue; Volkmann's canals, which run perpendicularly through the cortex bringing in blood vessels from the periosteal and endosteal surfaces and connecting adjacent Haversian systems; and the large medullary or marrow canal. With the exception of the canaliculae all of these spaces normally contain cells. The canaliculae are only partly filled by short cytoplasmic processes extended from the osteocytes.[4]

NUMBER OF SPACES IN BONE

Measurements made on material from a group of human cases of various ages in the Henry Ford Hospital Orthopaedic Research Laboratory are presented here. They were made on sections prepared by the writer's methods so no significant error occurs from shrinkage.[1,2]

There are from 4 to 25 vascular channels/mm^3 in human lamellar bone, there being characteristic variation with age, bone and part of a bone examined.[3]

There are about 26,000 osteocyte lacunae/mm^3 in human lamellar bone, there being about a 25% variation in this figure in different cases and ages. There are from two to three times this many lacunae in fibrous bone.[3]

There are about 40 canaliculae per osteocyte lacuna, baking a total of over 1,000,000 canalicular spaces/mm^3 in human lamellar bone and far more than this in fibrous bone.[3]

This is a truly impressive number of spaces in a cubic millimeter of bone. These spaces are so small that they normally comprise only about 7% of the total volume of the cortex, about 5% of the cortical volume being vascular canals and about 2% being the lacunar and canalicular lumens.[3]

BACTERIAL INVASION OF THE BONE SPACES

Now let us consider a portion of a bone which has been killed as the result of a pyogenic osteomyelitic process.[a] The dead bone portion may be cortical or cancellous and initially it is in physical continuity with adjacent, living bone. Only when repair processes intervene is a dead portion of bone separated from living. When separated it is called a sequestrum.

The vascular spaces in the dead portion of osteomyelitic bone contain multiplying pathogenic bacteria. Since the bone is dead, then by definition its cells are dead and constitute a good culture medium. The bacteria will multiply and grow into uninvaded dead tissue until all of this culture medium has been exhausted. The culture medium occupies every vascular channel and most of the lacunae. The result is that there may be bacteria present in about 1,000,000 spaces per mm^3 of the infected, dead bone. These bacteria may actually be photographed in situ in the bone spaces[3] in specimens from fresh cases of pyogenic osteomyelitis. We may accordingly consider the spaces in a dead, infected portion of a bone and the bacteria in them to be the *bacterial reservoir* of pyogenic osteomyelitis.

PERIOD OF VIABILITY OF THE BACTERIAL RESERVOIR

It is natural to inquire next about the length of time required for the bacteria in the bacterial reservoir described above to die as the result of exhaustion of their culture material. Here clinical experience is drawn on to provide an answer.

An attribute of osteomyelitis caused by beta hemolytic streptococcae and pneumococcae is the lack of recurrence once the immediate disease has run its course. This was true even in the preantibiotic era in patients who survived their initial infection. It is notable that pyogenic osteomyelitis due to these organisms is commonest in the first six months of life.

An attribute of osteomyelitis caused by staphylococcus aureus is its tendency to recur, often at some time remote from the initial osteomyelitic episode. Numbers of cases are known in which exacerbation occurred fifty years after the onset of the disease and the writer knows first hand of a case of Dr. Ward Plummer's of Buffalo[b] in which the recurrence was 70 years after the initial osteomyelitis.

If recurrences were due to reinfection of dead bone with new organisms by the hematogenous route, then the streptococcal and pneumococcal infections would also recur and there would be recurrences in which the organism was different from the

(a) It is understood that various factors in varying degree may be responsible for this local bone death. These factors would include venous thrombosis, increased hydrostatic pressure within the marrow cavity, direct bacterial invasion, action of bacterial toxins and accumulation of a large volume of exudate.
(b) Deceased 1952.

organism causing the initial ostemyelitic episode. Neither is the case. In fact, even strain types and antibiotic sensitivities of each recurrence are regularly identical to the types and sensitivities of the original organism, where this data is available. We may conclude that staphylococci in some manner can remain viable and virulent for many years in the bacterial reservoir. Similar behavior is distinctly unusual for streptococcae and pneumococcae.

DISCUSSION

The invasion of millions of tiny bone spaces by multiplying bacteria explains the source of the bacteria responsible for a recurrence of a pyogenic osteomyelitis. Questions are automatically raised as to the physical and chemical means of survival of staphylococci[c] over long periods in a portion of dead bone, the reason for the failure of other organisms to survive under apparently similar conditions, and the behavior of the large group of organisms which only occasionally cause pyogenic osteomyelitis but which have not been directly referred to in this paper. These are matters for additional work.

Another question occurs which is equally pertinent at this point, and that is: why do some cases of Staphpyogenic osteomyelitis recur and some not? In the hypotheti-

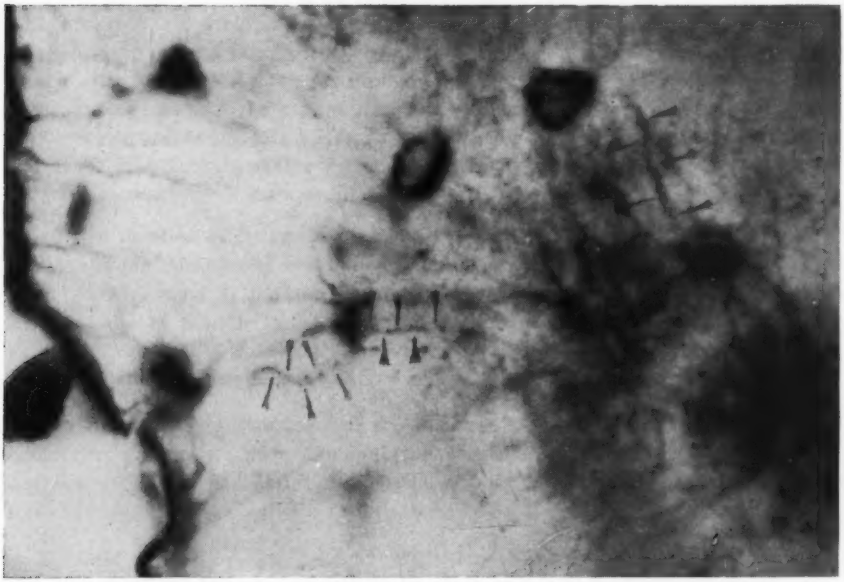

Figure 1

Cross section fibula 11 yr. girl with chronic osteomyelitis; surgically resected specimen. Fresh, undecalcified, fuchsin stain. The identifying black marks designate canaliculae within the depth of the section which contains minute, spherical particles that are probably staphylococci. The border of a resorption space is seen at the left of the figure. Such bacteria existing in the minute bone spaces over variable lengths of time constitute the "bacterial reservoir" of osteomyelitis.

(c) And perhaps some other organisms.

cal situation outlined, the factors stated were assumed to be constant from one case to another, leaving no room for the clinical variability which occurs. Additional factors such as the virulence of the organism, effectiveness of the host defense mechanisms, size of the dead portion of the bone, growth, remodelling activity and behavior of antibiotics in bone are known to exist and would be expected to influence the clinical course and outcome. Some of these factors will be considered in additional publications in preparation.

SUMMARY

Bone normally contains over 1,000,000 spaces/mm³. These spaces are heavily contaminated with bacteria in portions of bone killed during an osteomyelitic process. Clinical evidence suggests that the bacteria in these spaces constitute a *bacterial reservoir* which supplies the organisms responsible for recurrences of the osteomyelitis. The period of time during which the bacteria remain viable differs with different organisms, (and probably differs with additional factors not considered here), being longest for staphylococci and shortest for streptococci and pneumococci.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

2. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135 1959.

3. Frost H. M.: Previously unpublished work.

4 Weinmann, J. P., and Sicher, H.: Bone and bones, ed. 2, St. Louis, Mosby, 1955

LAMELLAR OSTEOID MINERALIZED PER DAY IN MAN*

H. H. Frost, M.D.

INTRODUCTION

The basic observation by Milch, Rall and Tobie that tetracyclines become fixed in and label newly mineralizing bone, and may subsequently be detected in undecalcified sections by microfluorescence,[14,15] has provided us with a potent new investigative tool to be used in the study of human bone physiology in vivo. Some aspects of the methodology involved with their use have been discussed elsewhere.[6,8,12,13] In this paper measurements of the thickness of new Haversian osteoid mineralized per day in a small group of patients will be presented. The measurements were made on undecalcified bone sections with the aid of tetracycline antibiotics given the patients at varying times before death or surgery.

When a patient receives tetracyclines, most of his body tissues become stained with the drug and remain so as long as the drugs are continued. Upon stopping the tetracyclines, the tissues destain. One exception to this destaining is the skeleton. Tetracyclines produce a peculiar stain at the outer edge of osteoid seams where progressive mineralization is occurring, and this stain is permanent in that it remains until the bone is resorbed by remodelling processes. Accordingly, bone which has been "labelled" with tetracycline in vivo at any time in the past contains the information of time, location, quantity and rate in its tetracycline label. This need only be read by suitable means after surgical resection or postmortem sampling to yield information on the osteoblastic activity of the patient during the time he was receiving the tetracycline antibiotic. In the Henry Ford Hospital Orthopaedic Research Laboratory, specimens are available which reveal the presence of tetracyclines given at known times and duration as much as 7 years prior to sampling of the skeleton.

If the duration of tetracycline administration is known, then it should be possible to measure the thickness of new bone being mineralized per day by dividing the width of the labelled band by the number of days over which the tetracyclines were given. It should also be possible to tell from such measurements whether or not there is any significant variation in rate of mineralization and whether or not the zone of tetracycline instantaneous staining is a sharp plane or a broad band.

In this paper measurements of the thickness of new, Haversian, lamellar bone mineralized per day are given. Circumferential lamellar bone formation is given in only one case (958200), and fibrous osteoblastic activity is deliberately excluded from this study.[7]

MATERIALS

Nineteen bones from 14 patients were examined. These patients all received tetracycline antibiotics at known times and for known duration prior to death or operation. The patients are listed in Table I along with other pertinent data. Patients known to have received androgenic, estrogenic or corticosteroid hormones have been excluded from this study, with the exception of case 854185 who received corticoids

*Work aided by Grant No. 293, Henry Ford Hospital Research Funds.

the last 3 weeks of life. Patients with moderate or severe congestive heart failure have also been excluded from this study. The reason for these exclusions is that osteoblastic activity as determined by several means is markedly affected by the hormones or disease state referred to and their effects on osteoblastic activity will be published separately.

CASE	Age	Sex	Duration Tetracycline dosage in days	Total average width tetracycline bands (u)	Range, width tetracycline bands (u)	Uncorrected osteoid mineralized per day (u)	Corrected osteoid mineralized per day	Spec. obtained OR or PM	Bones examined and # sections measured	Tetracycline given # months before PM or OR
198963	64	M	17	11.9	5-20	0.7	0.46	PM	Clav. 6	1.5
062070	56	F	15	18.7	9-25	1.25	0.98	PM	Clav.-Rib 6-6	1
243010	68	F	6	9.3	3-20	1.55	0.89	PM	Clav. 3	48
343203	70	M	5	7.3	3-9-12	1.4	0.66	PM	Rib 2	19
389217	61	F	10	14.7	5-30	1.5	1.07	PM	Clav.-Rib 3-2	72
529754	12	M	3	5.1	2-13	1.7	0.37	PM	Clav. 4	0.1
822604	35	F	5.5	5.9	2-9	0.98	0.35	O.R.	Rib 4	7
854185*	66	M	0.7	4.5	4-5	6.4	0.7	PM	Rib 4	0.03
			2	4.2	2-10	2.1	0.1		Clav.	20
855929	15	F	8	8.9	4-15	1.1	0.61	PM	2	11
890793	62	M	10	5.9	2-10	.59	0.2	PM	Clav. 3	0.3
954439	15	M	5	9.5	4-16	1.9	0.9	PM	Rib 4	0.6
958882	52	F	7	7.8	3-14	0.9	0.54	PM	Clav.-Rib 2-2	0.03
943014	57	M	11	24	5-55	2.2	1.8	O.R.	Fem-Tib-Fib 9	3.0
			18	39	15-60	2.2	1.9		Rib	1.7
958200	0.5	M	6	15.5	7-35	2.6	2.6	PM	3	0.9

Table I

METHODS

Fresh, undecalcified, complete cross sections of the bones examined were made, stained and mounted by the writer's methods.[1,2] The writer's simple microfluorescence set-up was utilized for visualization and measurement of the labelled bone.[6]

Measurements of the widths of tetracycline labelled bands in Haversian system (circumferential lamellae in case 958200) were made with a calibrated eyepiece micrometer at sufficient magnification and N. A. so that the only major error was estimation of the limits of the tetracycline band in the sections. An uncertainty of plus-minus 2

micra affects the latter estimation. Fifteen or more measurements on successive, labelled Haversian systems were made and averaged in each case. This average is tabulated in Table I as the average width of the tetracycline band. Measurements were not made in areas where the labelled structure was oriented obliquely to the optical axis of the microscope.

Time and duration of tetracycline dosage was obtained from hospital and OPD records.

The amount of osteoid mineralizing per day is obtained by division of the band width by the number of days of dosage.

OBSERVATIONS

From the data in Table I the following is either directly indicated or can reasonably be deduced.

From the range in width of the labelled bands in individual cases it appears that the rate of mineralization of new osteoid is a very variable thing and that this variation is a normal characteristic. We must assume that the osteoblastic activity which produces the osteoid being mineralized is subject to similar variation in rate. Seemingly some local factors exist which affect the rate of osteoblastic activity locally.

The average thickness of mineralized new osteoid per day for the whole group is about .9 microns. Considering the size of the group and the number of cases, the group differences obtained by averaging the results according to age, sex, and proximity in time to surgery or fatal illness are not significant. More cases and more measurements are needed before such comparisons would be statistically justified.

Case 854185 was included (in spite of corticoid therapy) because tetracycline was administered for less than a day. Yet the width of the tetracycline band in the labelled Haversian systems was 4.5 microns. This, plus comparison of the band widths in the other cases given tetracyclines for 3 days or less, indicates that at any one moment in time a band thickness of about 3-4 microns is being stained. This is the zone of tetracycline deposition. This zone moves centripetally a certain amount during each day of tetracycline administration. The instantaneous locus of the permanent tetracycline staining in mineralizing Haversian bone and circumferential lamellae is thus a 3-4 micron thick band, not an infinitely thin plane.

For the present, the writer adopts the value of 4 microns as the thickness of this instantaneous staining band. When more data are available this value may need to be increased or decreased by one micron or less. The value probably varies in individuals.

On the above basis Table I contains a column in which the corrected amount of osteoid mineralized per day is calculated. The calculation is simple: 4 microns are subtracted from the total thickness of the tetracycline band; the remainder is divided by the number of days of drug dosage.

Regardless of the value chosen for the momentary width of the tetracycline staining band, correction of the measurements for the value reveals that there are

considerable differences in values from patient to patient. These differences are too large to be errors in methodology. They reflect real variations among the patients.

DISCUSSION

It will be possible to calculate the volume turnover of bone, at least in diaphyses, by applying the above data to the geometry and to average measurements of diameter and length of Haversian systems in man, accounting in the calculations for the variation in numbers of Haversian systems with age and per unit area cross-section of bone and per unit volume of bone. Such determinations, being essentially numerical, will provide a good check on the planimetric methods used previously by the writer.[8]

Once we have good measures of normal bone remodelling activity, we may then apply these methods towards the investigation of osteoblastic activity and mineralization rates in various diseases, in particular senile and postmenopausal osteoporosis.

The measurement methodology outlined in this paper has potential as a means of investigating local and systemic factors controlling osteoblastic activity in both man and animal. The method is capable of considerable precision, something heretofore lacking in investigations of osteoblastic activity in man and animal. The reasons for previous lack of precision in the experimental and observational investigations of osteoblastic activity done to date are numerous. Included are the failure of previous workers with radioisotopes to recognize and correct for feathering,[5] average minerali-

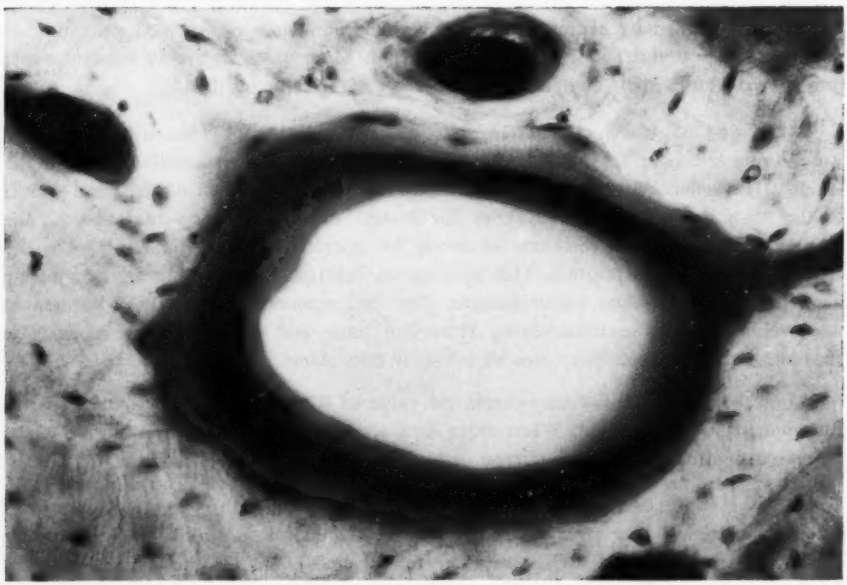

Figure 1

Clavicle; undecalcified, fuchsin stained section of patient who had received a tetracycline antibiotic up to 12 days before death. Administration of the drug then stopped so that in the 12 days preceding death, no tetracyclines were given. An osteoid seam lines the Haversian canal. Bright field.

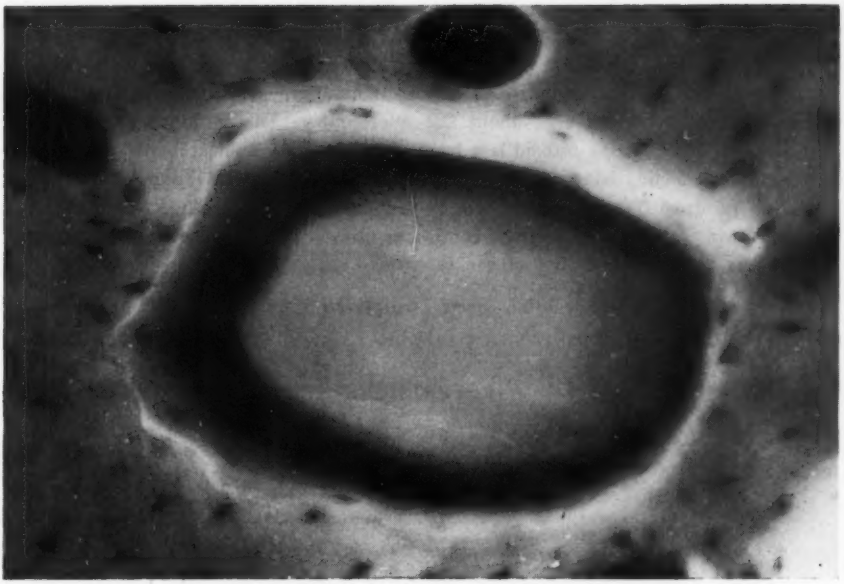

Figure 2

Same field but with writer's fluorescent set-up. An osteoid seam lines the central Haversian canal. At 9:00 o'clock it has grown 15 u centripetally from the bright band (the bright band is tetracycline fluorescing in already mineralized bone). The amount of osteoid mineralized per day in this part of the osteon is about 0.9 u when calculated as suggested in the test. At 12 and 1 o'clock the seam is in a resting state. There has been no significant mineralization of osteoid in this part of the seam in the 12 days before death of the patient. The seam lies directly on tetracycline labelled bone without any intervening unlabelled bone.

zation density, average cell death,,[3] normal distributional differential effects in man and animal[4,5,8,9] and failure to account for halo volume[9] and micropetrosis.[4] Measurements utilizing non-radioactive methodology have suffered from lack of technical development[8,11] and lack of knowledge of osteoblastic qualitative physiology.[5,7,8]

The variations in rate of mineralization of new osteoid illustrated in Table I are almost certainly the result of similar variations in rate of osteoblastic activity in the same zones. Such variations indicate the existence of local factors which affect osteoblastic activity in addition to the known (and postulated) systemic factors which affect—i. e.: regulate—osteoblastic activity. The methods used in this work would permit investigation of these local factors to be done, with the prospect that we might eventually learn how to make fractures in adults heal in weeks rather than in the months they do require. Such an objective will not be easily achieved, however! The reader should understand that initial fracture healing is accomplished by fibrous bone, and should realize that fibrous bone osteoblastic activity is subject to different local and systemic controls than lamellar bone osteoblastic activity.[7] Failure to make this distinction previously has seriously confused thought and work on the osteoblastic aspects of fracture healing, bone graft acceptance and the various forms of osteoporosis.

SUMMARY

Using in vivo tetracycline "labelling" of mineralizing, newly forming bone in a group of 14 humans, the thickness of osteoid mineralized per day has been measured on cross-sections and averages about 0.9 micron/day. The instantaneously staining part of newly mineralizing osteoid is a band about 4 microns thick. Tetracycline which is present outside this band during dosage with the tetracyclines disappears with cessation of the drug. Tetracycline present in this band is permanently fixed in place up to the 6 year period which was the maximum observed interval between dosage with the drug in vivo and sampling of the skeleton.

This is the first time it has been possible to measure directly an aspect of osteoblastic activity in man.

REFERENCES

1. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:272, 1958.

2. Frost, H. M.: Staining of fresh undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

3. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

4. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

5. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

6. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

7. Frost, H. M.: Observation on fibrous and lamellar bone, Henry Ford Hosp. M. Bull. 8:199, 1950.

8. Frost, H. M.: Measurement of bone formation in a 57 year old man by means of tetracyclines, Henry Ford Hosp. M. Bull. 8:239, 1960.

9. Frost, H. M., and Villanueva, A. R.: Measurement of osteoblastic activity in diaphyseal bone, Stain Technol. (In press).

10. Frost, H. M., and Villanueva, A. R.: Observations on osteoid seams, Henry Ford Hosp. M. Bull. 8:212, 1960.

11. Frost, H. M., Villanueva, A. R., and Roth, H.: Halo volume, Henry Ford Hosp. M. Bull. 8:228, 1960.

12. Frost, H. M., Villanueva, A. R., and Roth, H.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

13. Frost, H. M., Villanueva, A. R., and Roth, H.: Pyogenic osteomyelitis: Diffusion in live and dead bone with particular reference to the tetracycline antibiotics, Henry Ford Hosp. M. Bull. 8:255, 1960.

14. Milch, R. A., Rall, D. P., and Tobie, J. E.: Bone localization of the tetracyclines, J. Nat. Cancer Inst. 19:87, 1957.

15. Milch, R. A., Rall, D. P., and Tobie, J. E.: Fluorescence of tetracycline antibiotics in bone, J. Bone & Joint Surg. 40A:897, 1958.